Vol. XIII, No. 2 May, 1926

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF ECONOMIC BIOLOGISTS

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LONDON: FETTER LANE, E.C. 4
also
H. K. LEWIS & CO., LTD., 136, GOWER STREET, LONDON, W.C. 1
WHELDON & WESLEY, LTD., 2, 3, 4, ARTHUR ST., NEW OXFORD ST., W.C. 2
PARIS: LIBRAIRIE HACHETTE & CIE.
CHICAGO: THE UNIVERSITY OF CHICAGO PRESS
(AGENTS FOR THE UNITED STATES)
BOMBAY, CALCUTTA, MADRAS: MACMILLAN & CO., LTD.
TOKYO: THE MARUZEN-KABUSHIKI-KAISHA

Price Twelve Shillings net

PRINTED IN GREAT BRITAIN

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CONTENTS OF VOL. XIII, No. 2

PAGE

1. The Effect of Metallic Ions on the Growth of Hyacinths. By W. BLAIR BELL, M.D., B.Sc. and J. PATTERSON 157
2. Influence of Lead and the Metallic Ions of Copper, Zinc, Thorium, Beryllium and Thallium on the Germination of Seeds. By WALTER J. DILLING, M.B., Ch.B. (With 2 Text-figures) 160
3. Experiments on the Effects of Lead on the Growth of Plaice (*Pleuronectes platessa*). By W. J. DILLING, M.B., Ch.B., C. W. HEALEY, M.B., Ch.B. and W. C. SMITH. (With 3 Text-figures) 168
4. Influence of Lead and the Metallic Ions of Copper, Zinc, Thorium, Beryllium and Thallium on the Germination of Frogs' Spawn and on the Growth of Tadpoles. By WALTER J. DILLING, M.B., Ch.B., and C. W. HEALEY, M.B., Ch.B. (With 1 Text-figure) . . . 177
5. Notes on the English and American Races of the Greenhouse White-fly (*Trialeurodes vaporariorum*). By FRANZ SCHRADER. (With 5 Text-figures) 189
6. On the Early Stages of some Weevils (Curculionidae). By A. W. RYMER ROBERTS, M.A. Part I. (With 24 Text-figures) 197
7. Observations on the Biology of *Tylenchus dipsaci* (Kuhn) Bastian, and on the Occurrence of Biologic Strains of the Nematode. By W. E. H. HODSON, A.R.C.S. 219
8. On the Occurrence of *Centor myopinus* Meig. as a Pest on Oat. By W. E. H. HODSON, A.R.C.S. 229
9. Studies on Carbon Dioxide Production in Soil and Solution. By D. V. BAL. (With 2 Text-figures) 231
10. The Effect of some Soil Conditions on Nodule Formation on *Crotolaria juncea* (L.). By N. GANGULEE, Ph.D. (With 4 Diagrams) 244
11. The Organism forming Nodules on *Crotolaria juncea* (L.). By N. GANGULEE, Ph.D. . . 256
12. An Introductory Note on Two Bacteria causing an Internal Rot of Cotton Bolls. By J. C. HOPKINS 260
13. Notes on the Constitution of an Unimproved Cotton Crop. By B. G. C. BOLLAND, M.A. . 266
14. Concerning "Fairy Rings" in Pastures. By JESSIE S. BAYLISS ELLIOTT, D.Sc. (With 1 Text-figure) 277
15. Note on the Occurrence of Diseased Shoots arising from Potato-Tubers Infected by *Phytophthora infestans*. By Professor E. S. SALMON and W. M. WARE, M.Sc. (With Plate V) . 289
16. Further Experiments on the Use of Sulphur in Relation to Wart Disease of Potatoes. By W. A. ROACH, B.Sc., A.I.C., and WM. B. BRIERLEY, D.Sc. 301
17. Discussion on "The Fungicidal Action of Sulphur" 308
18. Report of the Council for the Year 1925 319
19. Report of the Hon. Treasurer for the Year 1925 321
20. Treasurer's Statement for the Year ending 31st December, 1925 322

VOLUME XIII MAY, 1926 No. 2

THE EFFECT OF METALLIC IONS ON THE GROWTH OF HYACINTHS[1]

BY W. BLAIR BELL, B.S., M.D. LOND. AND J. PATTERSON.
(*From the Department of Obstetrics and Gynaecology, Liverpool University.*)

IN order to test the effect of metallic ions on vegetable growth, a series of experiments was carried out on several hundred hyacinth bulbs.

All the bulbs, including the controls, were placed in the usual jars made for hyacinth bulbs after these vessels had been filled with solutions of the various metallic salts or ordinary tap-water. At first they were kept in the dark for about a fortnight, after which time they were moved to windows admitting a considerable amount of direct sunlight. The solutions, as well as the tap-water in which the controls were placed, were changed fortnightly.

The experiment was started at the beginning of October 1922, and by the time the bulbs were removed from the dark cupboard, all the controls had developed vigorous roots. The roots of the bulbs placed in contact with solutions of both normal and basic lead acetate of the strengths 0·1 per cent., 0·01 per cent., 0·001 per cent. and 0·0001 per cent. of lead ion[2] showed a graded degree of growth according to the strength of the solution. At the end of a month the controls showed signs of development of the leaves at the top of the bulb, and had strongly growing roots. Flowering began on February 1st, 1923, first in the controls. This development did not show quite the same gradation as was the case with regard to the appearance and growth of the roots. Nevertheless, there was a well-marked tendency among the bulbs placed in the stronger solutions of lead for the leaves and flowers to appear last; and, indeed, no flowers appeared in the bulbs placed in solutions of lead acetate of the strength 0·1 per cent. until March 12th.

A further experiment on the same lines was arranged; but in this second series, stronger and weaker solutions were used—that is, solutions

[1] This investigation was undertaken on behalf of the Liverpool Cancer Research Committee. Director: Prof. W. Blair Bell of the University of Liverpool.

[2] All percentages are in terms of the metallic ion.

of the strengths 1·0 per cent., 0·00001 per cent. and 0·000001 per cent. In these circumstances the differences of the root-growth were clearly shown: those bulbs that were placed in the 1·0 per cent. solution hardly developed at all; and in the weaker solutions there was gradation according to the strengths, in comparison with the growth of the controls. Flowering, however, was not so regularly distributed, but there was a very obvious difference, of course, between that taking place from the bulbs placed in the very strong and the weaker solutions. The flowers and leaves of the bulbs placed in the 1·0 per cent. solutions did not exceed 2 inches in height, whereas those of the bulbs in the weakest solutions, and the controls, grew to a height of approximately 12 inches.

A third series, in which 54 hyacinths were used, was also investigated. In this experiment solutions of the same salts in strengths of 0·1 per cent., 0·01 per cent., 0·001 per cent. and 0·0001 per cent. were employed and confirmatory results were obtained. It became obvious, however, that in the weakest solutions although the root-growth was considerably less than that of the controls, the leaf and flower formations could not definitely be distinguished from the flowers and roots of the control bulbs. When, however, the solution was strong—0·1 per cent. or 1·0 per cent.—not only was the root-growth completely arrested, but also the flowers and the leaves remained stunted to a remarkable extent. Between this strength and that of the weakest solutions, the root-growths were accurately graded, but this did not obtain in regard to the flowers and leaves of the bulbs.

The following autumn (1923) the same bulbs were grown again, but in tap-water only, and it was found that those which had been immersed in lead solutions in the previous year were stunted in growth, both as regards the roots, flowers and leaves. Indeed, as regards the roots, they exactly reproduced the appearance seen the previous year, and in regard to the flowers and leaves, these gave more definite indication of conformation with the amount of root-growth than had been observed previously.

On chemical examination, two of the bulbs that had been placed a year previously in lead solutions were found to contain lead. This may be taken as indicating that the bulbs had actually taken up lead in close association with some chemical element in their constitution. It appears, therefore, fair to assume that lead has some effect on the chemical factors concerned in growth, probably on the function of the phosphatides present.

Other experiments were conducted to investigate the effect of salts of calcium, copper and zinc on the growth of hyacinth bulbs.

Certain effects on growth were produced; but these were not really comparable with the effects of lead. Zinc and calcium appeared to be very toxic in the stronger solutions—that is, solutions containing from 0·001 per cent. to 1·0 per cent. of the metallic salts. Root-growth was practically absent, and in only one case did flowering occur. In the weakest solutions the root-growth and flowering were not affected.

The same results were obtained with calcium salts.

Copper was found to be extremely toxic, and in solutions the strengths of which were as low as 0·000001 per cent. growth of the plant was entirely prevented.

It appears likely, therefore, that zinc and copper are directly poisonous, and do not produce a true graded effect of stunting in various solutions: they appear either to kill or not to interfere with growth at all.

Calcium probably acts by reducing the permeability of the cell membranes.

We have not yet carried the experiments further to note whether the bulbs which showed no signs of growth were killed and would not develop subsequently. It seems probable that bulbs poisoned by zinc and copper to such an extent as to prevent all growth would not be likely to grow the following year. Whereas calcium should only produce a temporary effect.

(*Received November 6th*, 1925.)

INFLUENCE OF LEAD AND THE METALLIC IONS OF COPPER, ZINC, THORIUM, BERYLLIUM AND THALLIUM ON THE GERMINATION OF SEEDS[1]

By WALTER J. DILLING, M.B., Ch.B.
(*From the Department of Pharmacology, Liverpool University.*)

(With 2 Text-figures.)

The experiments which follow form part of a comprehensive investigation by the Cancer Research Committee of Liverpool University on the inhibiting and sometimes absorptive influence which lead appears to exercise on certain types of malignant neoplasms (1). It was argued that if lead did exert an inhibitory effect on the growth of a rapidly proliferating pathological tissue it would probably at least delay the normal processes of germination and growth in plants.

The experimental work of Brenchley at Rothamsted Experimental Station has shown that extremely minute traces of copper, zinc and arsenic have a deleterious action on the growth of peas and barley in presence of nutrient salts. She found that copper sulphate in 1 : 1,000,000 concentration entirely checked root growth and suggested that the absorption layer of the root, acting as a semi-permeable membrane and upheld by the resistant protoplasm, was able to keep the copper out of the plant if the solution was dilute enough. After a certain limit, however, the copper exercised a corrosive influence on the outer layers of the root which became unable to resist entry of the poison and growth was partly or entirely checked (3). Miyajima has stated that 1 per cent. copper solutions partly destroy the germinating power of the seeds of *Vicia Faba*, *Pisum sativum* and *Zea Mays*, but Miani, on the other hand, found that traces of copper did not hinder germination of pollen grains or spores. Storp found, in experiments with zinc oxide (0·09 per cent.), that germination of maize was retarded but growth of barley was uniform

[1] This investigation was undertaken on behalf of the Liverpool Cancer Research Committee. Director: Prof. W. Blair Bell of the University of Liverpool.

while the seeds were kept in the dark, but was retarded when the young plants were exposed to light, which indicated that zinc impaired the activity of chlorophyll. Brenchley found zinc sulphate in a nutrient salt culture to be deleterious to the growth of pea plants in dilutions of 1 : 100,000 (3).

The following series of experiments was done with the object of ascertaining whether lead salts had any special retarding influence on the germination and early growth of seeds and whether any other metals possessed the action in more marked degree. The experiments were done chiefly with cress (*Lepidium sativum*) and mustard (*Sinapis alba*) seeds. Sunflower seeds and peas were also employed and gave results which agreed in type with the others, but these seeds were abandoned after several trials as they failed to grow well in a city laboratory.

Method. Attempts were made, at first, to ascertain the effects of lead water on seeds planted in soil, but the results were found to be inconstant and unreliable, although generally confirmatory of the findings to be quoted. On the assumption that part of the lead was adsorbed or precipitated by the soil ingredients, cultures of sand and various saw-dusts were tried, but with little advantage.

The method found most satisfactory was to place the seeds on a piece of jute sacking or loose-wove muslin tied over a flat cork, or latterly a cork ring, which floated on the leaded water; capillary attraction kept the jute moist and the roots could penetrate through the open meshes. The corks floated in beakers covered with dark paper and evaporated water was replaced as necessary.

The following is a typical result in synopsis:

Table I.

Cress Seeds in Lead Acetate calculated as Pb *Ion.*

50 seeds in each test.

Day after planting	4th day	5th day	8th day	10th day	19th day
Control	Growth started	Stems $\frac{1}{4}''$	Stems $\frac{1}{2}''$	Stems $1''$	40 germinated. Growth $1\frac{1}{2}''$, prim. and second. leaves
Lead 0·05 %	—	Just bursting	Stems $\frac{1}{4}''$	Stems $\frac{3}{4}''$	32 germinated. Growth $1\frac{1}{2}''$, prim. and second. leaves
„ 0·12 %	—	—	Growth started	Growth slow	19 germinated. Growth $1''$, weak, prim. and second. leaves
„ 0·27 %	—	—	—	—	0 germinated, except 2 small rootlets
„ 0·5 %	—	—	—	—	No growth

Table II.

Mustard Seeds in Lead Acetate calculated as Pb *Ion.*

50 seeds in each test.

Day after planting	4th day	5th day	8th day	12th day	19th day
Control	Growth started	Stems $\frac{3}{8}''$	Stems $\frac{3}{4}''$	Stems 1–1$\frac{1}{4}''$	24 germinated. Growth 1$\frac{1}{2}$–2″, prim. and second. leaves
Lead 0·05 %	—	Growth started	Stems $\frac{1}{2}''$	Stems 1″	15 germinated. Growth 1$\frac{1}{2}$–2″, prim. and second. leaves
,, 0·12 %	—	—	Growth started	Stems $\frac{1}{4}''$	8 germinated. Growth $\frac{1}{2}$–1″, cotyledons only
,, 0·27 %	—	—	—	One root showing	1 germinated. Growth $\frac{1}{8}$–$\frac{1}{4}''$, cotyledons only
,, 0·5 %	—	—	—	—	No growth

It will be seen that the lead has a definite inhibiting action on germination and, in strengths of 0·27 per cent., practically checks it, whilst it also exercises a deleterious effect on the growth of the seedling. With 0·12 per cent. germination is delayed by four days, is on an average 40 per cent. below the control and the growth is slow and stunted; 0·05 per cent. reduces germination by about 20 per cent., delays it by a day, but the young plant makes good growth. In Tables III and IV are the abbreviated notes of the series from which Figs. 1 and 2 were obtained on the 28th day after planting.

Table III.

Cress Seeds in Lead Acetate calculated as Pb *Ion.*

50 seeds in each test.

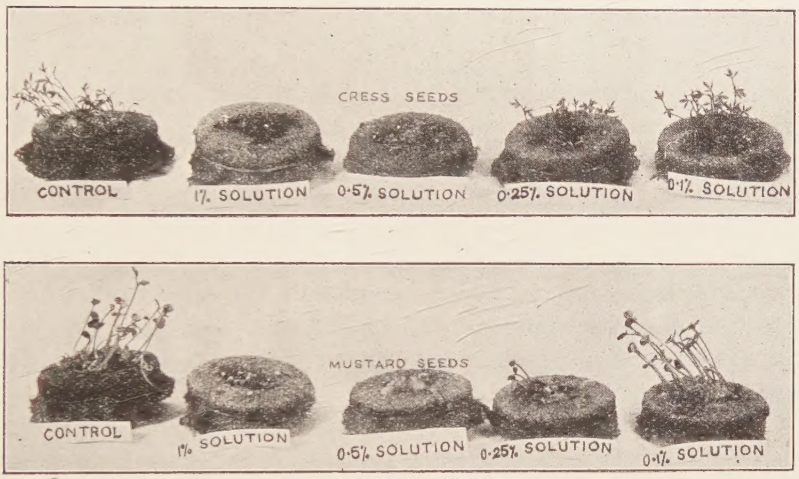
Day after planting	3rd day	8th day	10th day	25th day	45th day
Control	Roots visible	Stems $\frac{1}{2}''$	Thick growth $\frac{3}{4}''$	Strong growth 1″	42 germinated. Growth 1$\frac{1}{2}''$, prim. and second. leaves
Lead 0·05 %	Roots visible	Stems $\frac{1}{2}''$	Thick growth $\frac{3}{4}''$	Strong growth 1″	36 germinated. Growth 1$\frac{1}{2}''$, prim. and second. leaves
,, 0·12 %	A few roots visible	No progress	No progress	Stems $\frac{1}{8}''$	16 germinated. Growth stunted, $\frac{1}{8}$–$\frac{1}{2}''$
,, 0·27 %	A few roots visible	No progress	No progress	Stems $\frac{1}{8}''$	6 germinated. No growth
,, 0·5 %	—	—	—	—	0 germinated. No growth

Table IV.

Mustard Seeds in Lead Acetate calculated as Pb *Ion.*

50 seeds in each test.

Day after planting	3rd day	7th day	12th day	22nd day	40th day
Control	Roots visible	Stems $\frac{1}{4}''$	Stems $\frac{3}{4}''$	Stems $1–1\frac{1}{4}''$	22 germinated. Growth $1\frac{1}{2}''$, prim. and second. leaves
Lead 0·05 %	Roots visible	Stems $\frac{1}{8}''$	Stems $\frac{1}{2}''$	Stems $1–1\frac{1}{4}''$	18 germinated. Growth $1\frac{1}{2}''$, prim. and second. leaves
,, 0·12 %	Roots visible	No progress	No progress	Stems $\frac{1}{8}''$	4 germinated. Growth stunted, $\frac{1}{8}–\frac{1}{2}''$
,, 0·27 %	—	—	—	—	0 germinated
,, 0·5 %	—	—	—	—	0 germinated.



Figs. 1 and 2. Inhibition of germination or retardation of growth on 28th day of cress and mustard seeds grown on jute-covered corks floating on lead acetate solutions of various strengths. (N.B. Corresponding strengths of lead as ion are 0·5 %, 0·27 %, 0·12 % and 0·05 % respectively.)

In the next series (Table V) growth was represented by the fraction of number of seeds showing $\frac{\text{roots}}{\text{cotyledons}}$. It shows again that by progressive increase in lead ion the actual germination is inhibited (absence of root) and also the delay in subsequent growth (absence of cotyledons). The growth in the higher concentrations was always pale and stunted.

Comparison of the tables shows that, in most cases, between 0·15 and 0·2 per cent. lead ion is required to inhibit seed germination; this represents a lead acetate solution of 0·3 to 0·4 per cent., very high percentages and much above those proved to be toxic to young plants

in the case of copper and zinc. Very probably lead in much weaker solutions would interfere with the growth of pea seedlings grown under similar conditions.

Table V.

Lead Nitrate on Growth of Root and Cotyledons of Cress Seeds (Sand Culture).

10 seeds in each test.

	3rd day	4th day	8th day	11th day
Control	9/1	9/4	10/10 (1″)	10/10 (1½″)
Lead ion 0·001 %	7/0	8/6	8/6	8/8 (1½″)
„ 0·01 %	6/0	7/6	8/7	8/8 (¾″)
„ 0·1 %	3/0	4/0	6/4	7/5 (⅛″)
„ 1·0 %	0/0	0/0	0/0	0/0

Further experiments were done in order to ascertain whether ionic lead had a permanent effect in stunting or retarding the growth of these seeds; it was found that, although the lead delayed germination and initial growth, the young plants recovered on being transferred to fresh water and the secondary leaves formed about 6 days after those of the control, in fact, in two series, the first in lead ion 0·12 per cent. for 6 days and the second for 8 days, the growth was equal to that of the control on the 14th and 19th day respectively. Lead ion, therefore, delays growth but does not cause permanent injury to the germinating tissue of seeds.

This point was brought out also by an experiment on cress seeds which had failed to germinate when kept for 18 days on jute moistened with 0·07 per cent. lead ion and 0·15 per cent. lead ion. Ten seeds from each were then placed on washed sand and the remainder left in contact with the metallic solution as controls of absence of growth. The following table shows the result in $\frac{\text{root}}{\text{cotyledon}}$ formation and in size:

Table VI.

Growth of 10 Cress Seeds transferred to Water Culture after failing to germinate in Lead ion in percentages quoted.

	Lead ion 0·07 %	Lead ion 0·15 %
1st day	0/0	0/0
2nd „	6/0	6/0
5th „	6/5 (1″)	9/6 (1″)
8th „	7/6 (1½″)	9/8 (1½″)

Lead ion can, therefore, inhibit, without necessarily destroying, capacity for germination.

This observation is of interest, when one bears in mind that, in animals and humans, chronic lead poisoning frequently causes death of the germinating ovum in the uterus without, however, influencing deleteriously the unmatured ova in the ovary. The cases are not strictly comparable except that in both instances lead seems to inhibit growth without killing the potential or latent germinal force.

It was next sought to determine whether this was some specific action of lead or whether there were differences between its action and that of other metals. The metals tested were zinc, thorium, beryllium, copper and thallium, all being utilised in the form of the soluble nitrates and the concentrations being prepared from "deci-normal" solutions so that the free metallic ions in corresponding dilutions were identical. The results may be summarised thus: thorium ion delays germination and growth nearly to the same extent as lead; zinc and beryllium have less effect than thorium; copper ion does not seem to have the same power as lead to inhibit germination but it has more power to retard the subsequent growth of the plant; thallium ion proved to be the most potent in checking both growth and germination. The *p*H of the solutions was tested but did not appear to be the determining factor in the effects noted nor did the molecular concentration of the salt have any relation to the comparative action of the metals. It would, therefore, appear that other metallic ions possess, like lead, the power of inhibiting germination and growth of seeds.

Table VII.

Effects of different metals on Germination and Growth of Cress Seeds at 18th day after Planting.

20 seeds in each test.

Strength	Water control	*N*/600	*N*/300	*N*/150	*N*/75
Lead	19/19 (1″)	11/7 (½″)	10/3 (⅛″)	5/0 (⅛″)	0/0
Thorium	18/18 (1″)	14/8 (½″)	9/6 (¼″)	4/1 (⅛″)	0/0
Zinc	17/17 (¾″)	10/9 (⅜″)	16/14 (¼″)	16/4 (⅛″)	13/2 (⅛″)
Beryllium	20/20 (1″)	19/11 (½″)	13/7 (½″)	16/8 (¼″)	13/7 (¼″)
Copper	18/18 (¾″)	12/3 (¼″)	10/1	6/0	9/0
Thallium	18/18 (1″)	2/0	5/0	2/0	0/0

In a subsequent series of tests, it was ascertained that seeds which had failed to germinate when in contact with various metallic nitrate solutions for 18 days would also germinate and grow, if transferred to a float on unpoisoned water. This was so in seeds treated with zinc and

beryllium; these grew practically at the normal rate on transfer. The thorium series showed slight delay. Those which had been in copper salts also germinated but, whereas the young plants were 1 to 1½ in. long eight days after removal from the lead, zinc, thorium and beryllium solutions, those of the copper series reached only ⅛ in. in the same time and progressed no further. Thallium ion proved to be a still more potent poison and the seeds failed altogether to germinate on transfer.

General Summary.

Cress and mustard seeds were particularly suited for these experiments as neither is endowed with much reserve food supply. Practically speaking they have to form their own food from the moment the seed opens, when the cotyledons develop chlorophyll and perform the functions of true leaves. While recognising the wide difference between the chemical composition and physiology of germinating tissues amongst plants and animals, it was thought possible that the effect of lead on the germination of such seeds might throw some light on its power of causing abortion in mammals, if, for example, this were by toxic action on embryonic tissues, and that the more resistant vegetable cells might exhibit marked differences in susceptibility to metallic ions which might afford clues for further investigations on vertebrate spawn and mammals. The experiments did not, however, demonstrate marked differences between lead and other metallic ions tested. They proved, nevertheless, that seeds are remarkably resistant to these metallic poisons and observation has shown that the seeds will imbibe moisture and swell in a poisonous solution but will avoid extruding a root; in other instances a tiny radicle may just be pushed out only to remain dormant in the hostile medium; or, as has been noted in the higher concentrations, the tiny radicle may show negative heliotropism and attempt to rise from the poisoned solution into the wholesome moist air. This last phenomenon was seen most frequently when cultures were protected from surface evaporation by glass capsules. Darwin's comparison of the root-tip to a brain recurred to one in observing these experiments which have also shown what extraordinary powers seeds possess of surviving adversity. When removed from strengths of metallic salts which it might be presumed had been fatal to their germinating power, they immediately exhibit vitality and rapidly make up for the enforced delay in their germination. From familiarity with the obstinacy of seeds to germinate in unsuitable soils, one would be inclined to ascribe this to reflex inhibition of germination in a hostile medium. One feels that, if there had

been any corrosive action or toxic effect from penetration of the metallic ion through the cellulose walls into the embryo, the subsequent growth would have been stunted, but this was observed only in the case of copper salts. Certainly, no evidence of "corrosive" effect on the radicles was observed. As the inhibitory effect is a reversible one, there is ground for suggesting that the effect may be one of colloidal adsorption of lead on the superficial growing cells—their germinating powers being thus temporarily inhibited, although not killed—and that, on transfer to a watery medium, the adsorbed lead redissolves and leaves the uninjured cells free to continue proliferation.

The results led to tests being made of copper, thorium and thallium salts on vertebrate spawn and, in order to ascertain their powers of inducing abortion, on pregnant rabbits. It was found, however, that, while lead salts were active abortifacients by a toxic action on the foetal ectoderm (trophoblast) without affecting the maternal organism in the doses used, copper salts caused abortion through foetal haemorrhage, were uncertain in effect, and deleterious to the mother; thallium salts had uncertain abortive effects due to haemorrhages; and thorium was atoxic to the chorionic epithelium (2).

Conclusions.

(1) Lead salts in concentrations above 0·01 per cent. lead ion delay the germination and growth of seeds (cress and mustard).

(2) Lead salts in concentrations of about 0·1 to 0·2 per cent. lead ion can inhibit germination of cress and mustard seeds for at least 18 days without destroying the vitality of the seeds, which will still germinate on transfer to a water culture.

(3) These actions are not characteristic or specific of lead. Thorium, zinc and beryllium salts in equivalent molecular concentrations give similar but less marked effects; copper stunts the subsequent growth of inhibited seeds; while thallium seems to destroy the vitality of the seed.

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(*Received November 6th*, 1925.)

EXPERIMENTS ON THE EFFECTS OF LEAD ON THE GROWTH OF PLAICE (*PLEURONECTES PLATESSA*)[1]

By W. J. DILLING, M.B., Ch.B., C. W. HEALEY, M.B., Ch.B. and W. C. SMITH.

(*From the Department of Pharmacology, Liverpool University.*)

(With 3 Text-figures.)

The experiments to be discussed below were carried out at the Biological Station, Port Erin, Isle of Man, where tanks were kindly placed at our disposal by the late Sir Wm. Herdman and Prof. Johnstone. After the preliminary arrangements were set up, the subsequent control of the treatment and measurements were carried out by Mr W. C. Smith, Curator of the Station.

I. Experiments with Plaice Eggs.

A solution of lead nitrate was prepared with sea water; a heavy white precipitate of lead carbonate and phosphate resulted. It was considered that the addition of such a milky fluid to the tanks would not be advisable since the precipitate would certainly interfere with the respiratory function by clogging the gills. The precipitate was filtered off and the clear filtrate was found to contain free lead ion when tested by hydrogen sulphide.

Method. The plaice eggs were divided into four batches which were placed in the middle two of the four compartments of the hatching troughs. Into the first compartment fresh sea water flowed at the rate of 27·6 c.c. per minute and into this, at the inflow, the lead solution was allowed to drip at a definite rate. Subsequent chemical estimation showed that the eggs in Series A lay in a 0·00005 per cent. lead ion solution; and Series B in $\frac{1}{10}$ this strength (1 in 20,000,000): Series C and D were controls. The *p*H of the water, corrected for salt error, was found to vary only from 8·1 to 8·3.

[1] This investigation was undertaken on behalf of the Liverpool Cancer Research Committee. Director: Prof. W. Blair Bell of the University of Liverpool.

The experiment started on May 2nd when the eggs were 9 days old; *i.e.* embryos being still within the egg capsule. Later the rate of lead inflow was increased and on May 8th the lead content of the water of Series A was 0·0004 per cent. (1 in 250,000) and on May 22nd, when the experiment ceased, the water in the tank contained 0·00016 per cent. of lead ion (1 in 600,000) by chemical test.

Metamorphosis of the eggs took place normally and although specimens were taken and examined microscopically in the intact state and in sections, no difference in development between the controls and "leaded" specimens could be found. During the experiment, heavy casualties occurred—chiefly about the 14th day when the food of the yolk sac was absorbed and the young plaice commenced to feed on Diatoms and Copepoda. It was noted that the more heavily "leaded" Series A fish survived longer than the others. That this might be due to a delay in the attainment of the independent state was suggested, but microscopical examination did not afford proof that this was the explanation.

This result is of a negative character; it indicates that in the homoeopathic doses in which lead ion can exist in sea water it has no specific or clearly demonstrable action in delaying the germination of plaice eggs.

II. Experiments on Young Plaice.

Forty-four young plaice were measured in millimetres and divided into three series and placed in 5000 c.c. sea water. To the water of Series A, 10 c.c. of 0·2 per cent. colloidal lead was added, of Series B, 5 c.c. of 0·2 per cent. colloidal lead, while Series C was kept as a control. The waters were changed every second day and the fish were measured every month. The "leading" commenced on June 19th, 1924, and ceased on November 22nd—a period of 5 months. The amounts of colloidal lead were purposely kept very low because it was particularly desired to avoid all possibility of injury or speedy toxic effect on the young fish, but to ascertain the immediate and, if possible, remote action of traces of colloidal lead on growth; *i.e.* whether the effect on the rate of development would be transient or permanent.

The colloidal lead used consisted of colloidal metallic lead containing colloidal lead hydroxide; the largest particles of which were 0·2μ. It is unlikely, therefore, that the colloid interfered with the respiratory function of the gills as would almost certainly occur if high concentrations of ionic lead salts were used, or if a precipitated salt were suspended

in the water. The *p*H of the lead solution was 10·3, a negligible factor with the small amounts utilised.

The figures in Table I show the average lengths of the fish in the three series over the period of a year; it should be noted that the figures subsequent to June 1925 cannot be considered reliable, as casualties due to the heat-wave altered the averages.

Table I.

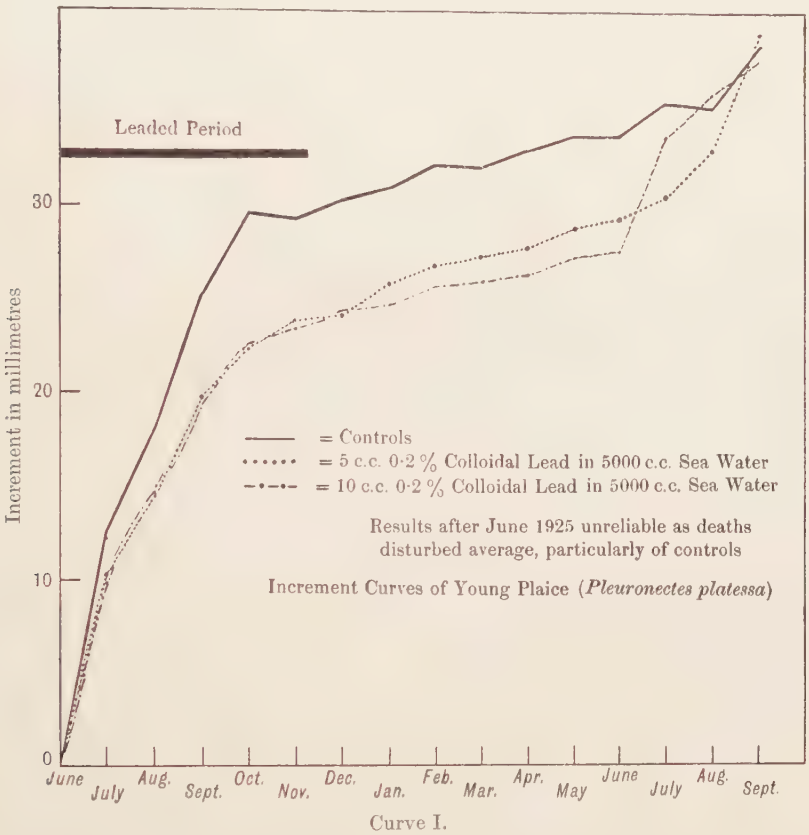
Average Lengths in millimetres of Young Plaice under experiment with Colloidal Lead.

		Controls in 5000 c.c. sea water			Series B 5000 c.c. + 5 c.c. 0·2 % coll. Pb			Series A 5000 c.c. + 10 c.c. 0·2 % coll. Pb		
		Mean sizes	Mean growth in month	Increment from June	Mean sizes	Mean growth in month	Increment from June	Mean sizes	Mean growth in month	Increment from June
Leaded period	June	19·47	—	—	17·60	—	—	15·79	—	—
	July	31·80	12·33	12·33	27·80	10·20	10·20	25·57	9·78	9·78
	Aug.	37·57	5·77	18·10	32·07	4·27	14·47	30·21	4·64	14·42
	Sept.	44·75	7·18	25·28	37·36	5·29	19·76	35·07	4·86	19·28
	Oct.	49·00	4·25	29·53	39·92	2·56	22·32	38·15	3·08	22·36
	Nov.	48·83	−0·17	29·36	41·41	1·49	23·81	39·23	1·08	23·44
	Dec.	49·67	0·84	30·20	41·75	0·34	24·15	40·08	0·85	24·29
	Jan.	50·33	0·66	30·86	43·55	1·80	25·95	40·54	0·46	24·75
	Feb.	51·75	1·42	32·28	44·45	0·90	26·85	41·58	1·04	25·79
	March	51·64	−0·19	32·17	44·82	0·37	27·22	41·75	0·17	25·96
	April	52·54	0·81	32·98	45·36	0·54	27·76	42·17	0·42	26·38
	May	53·20	0·75	33·73	46·55	1·19	28·95	43·08	0·91	27·29
	June	53·20	—	33·73	47·00	0·45	29·40	43·41	0·33	27·62
	July	55·00	1·80	35·53	48·12	1·12	30·52	49·57	6·16	33·78
	Aug.	54·80	−0·20	35·33	50·71	2·59	33·11	51·71	2·14	35·92
	Sept.	58·00	3·20	38·53	62·00	11·29	44·40	55·00	3·29	39·21

The resultants of the table are illustrated in Curve I which shows the increment in growth of the three series; curves plotted to show the monthly growth averages and mean growth exhibit the same characteristics.

In the curve it will be seen that the more heavily leaded fish exhibit distinct retardation of growth as compared with the controls, while the curve of the less leaded series occupies an almost identical position. Attention is drawn to the fact that the most marked effect occurs while the fish are in the leaded water and that, after removal from the lead, the growth curves follow closely the seasonal variations of the normal curve and gradually approach the normal. This result would indicate that, while lead delays growth, its deleterious effects soon pass off when the animal is removed from the lead environment. This conclusion cannot,

however, be reached with certainty, since the lead was withdrawn in the late autumn, at about the time when growth normally begins to slow off considerably, with the natural result that, during the following six months, little growth was shown by any of the series. Then, when accelerated growth was to be expected again in the following June, rather heavy casualties, caused by the heat-wave during that month,

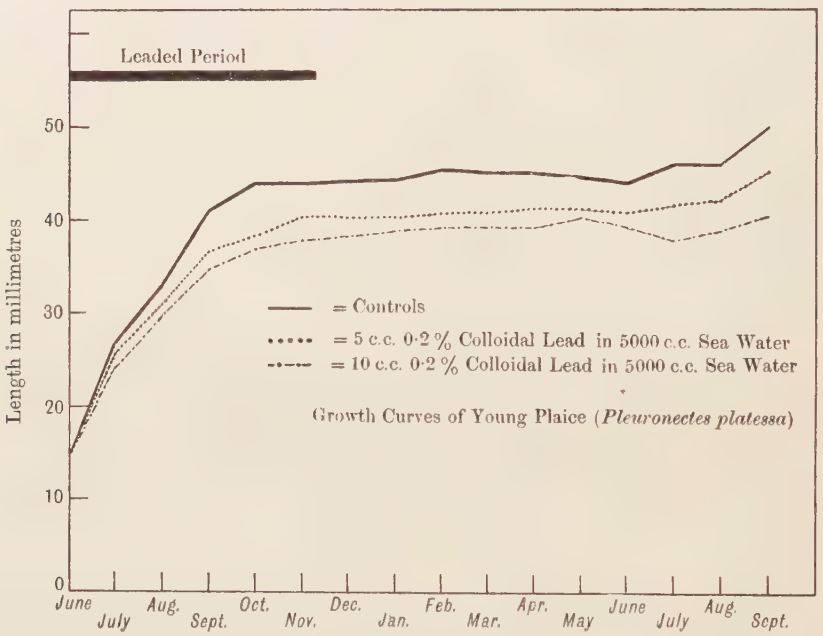


Curve I.

greatly affected the comparative value of the averages. The casualties were chiefly amongst the smallest fish of the "leaded" series and the largest fish of the controls, so that the final control figures are probably much too low in comparison.

It is usual for young plaice to become sluggish during the winter months when the water is cold, but to show signs of a certain "liveliness" occasioned by increasing vitality when the temperature rises in June.

In the present case, the revival proved fatal to certain of the larger fish of the controls, which jumped out of the tanks during the night and were lost. All the leaded fish which became casualties at this time died in the tanks when a sudden rise of temperature occurred early in June. As the controls suffered no loss from this cause, it would seem that the vitality of the small leaded fish had, to some extent, been impaired for at least seven months after contact with the lead. It is noteworthy that the losses sustained, presumably through excess of vitality, in the control series, occurred in the larger sizes, and that the leaded fish which



Curve II.

died in the tanks were mainly confined to the smaller individuals. Comparison of the growth curves of the individual fish supports the contention that the "leaded" fish are in proportion to the amount of "leading" definitely retarded in growth. We noted early in the experiment that by chance the fish when first measured had been divided into series which gave the controls on calculating the mean length a "start" above the leaded series. Curve II shows the growth curves of fish which were all 15 mm. in length at the commencement; the retardation of growth in proportion to the leading is here apparent.

Mention may here be made of certain relevant points from the observations of others. Miss Carpenter has pointed out the paucity of fauna in streams polluted by lead mines in Wales. She records the observation of Rushton (4) that lead nitrate 1 in 100,000 is fatal to trout in $2\frac{1}{4}$ hours and rightly contends that this proportion is relatively high and that, in experiments with fish, no cognisance has been taken of their invertebrate food organisms. She considers that a diffusible lead content up to 0·1 mgm. per litre (1 in 10,000,000) is no barrier to establishment of a fairly rich invertebrate fauna, but fish apparently do not live in this concentration (1). In the experiments here quoted the lead was utilised in purely colloidal form in strength of 1 in 250,000; retarded growth of the fish occurred, but, from our observations on paramoecium, this strength is too attenuated to kill lower organisms in the course of a day at least, hence we are forced to the conclusion that the retarding effect of lead on the growth and vitality of the fish was the direct deleterious action of the lead on the growth of the animal and not a starvation effect. Morgan (3) speaks of fish in streams polluted by Welsh lead mines as dying from interference with the respiratory function of the gills. Such cases can be readily understood, although Miss Carpenter believes them to be uncommon (1). Our experience with colloidal lead containing particles no greater than 0·2 μ shows that interference with growth occurs even with quantities insufficient to kill the fish and, we believe, its food, or interfere with its respiratory function through precipitation of protein on the gills and that the set back in early life tends to persist.

In her recent paper Miss Carpenter found that minnows and trout both in river cages and in the laboratory succumb in about 12–36 hours to lead in concentration of 0·3 to 0·4 mgm. per litre (*i.e.* about 1 in 3,000,000) and show post-mortem a film of precipitated mucus over the body and gill filaments. She further stated that, while minnows will survive in a 1 mgm. per litre Pb (1 in 1,000,000) for 13 days when the solution is not changed, they die in 48 hours if the solution be frequently renewed. Experiments on goldfish showed that 10 mgm. Pb per litre (1 in 100,000) as the acetate was tolerated if the solution were not renewed, but was fatal in 12 days if renewed on alternate days. She concluded that the fatal effect was not due to the nature or strength of the lead salt but to the actual quantity of lead available (2).

The extreme dilutions with which she obtained poisoning in minnows and trout possibly indicate that these fish are unusually susceptible to lead. We record here the experiments of one of us (C. W. H.) on goldfish. These were done with the object of ascertaining if lead would influence

the weight curve—rapid poisoning was not the object. Instead of 500 c.c. water, 6000 c.c. of water were employed with six goldfish in each bowl, the solutions were changed daily and ample food material supplied. To one bowl 3 c.c. $N/1$ lead nitrate was added (*i.e.* Pb 1 in 20,000); three fish were dead in 24 hours, the remaining three sluggish except for exaggerated movements of the operculum, indicative of respiratory distress; these three died in the course of the next 24 hours. All these fish showed a film of adherent precipitated protein.

The second bowl contained 3 c.c. $N/10$ lead nitrate (*i.e.* Pb 1 in 200,000); one fish died after 22 days, four died at intervals during the second month, and one survived three months in the solution. Two fatalities—one accidental—occurred in the control series during the third month of the experiment. The effect on the weight curve of the fish in this experiment, although definite, was not considered sufficiently striking to merit fuller investigation for our purposes; it was found that reduction of weight occurred also in the controls, but that the average ratio was in one month 10 per cent. reduction in the fish in 1 in 200,000 Pb, while that in the controls was only 5 per cent. One point noted was that the weight of the fish when found dead was valueless—it was invariably much increased—due perhaps both to swallowing of water prior to death and to imbibition of water after death.

The survival of the goldfish in large quantities of 1 in 200,000 lead solution changed daily for over a month does not lend support to Miss Carpenter's suggestion that the fatal effect depends "not on strength of lead salt but rather on the actual quantity available." Our goldfish had ample lead available for absorption, but in such dilution that it did not cause gross toxic effects; in other words, 1 in 200,000 is below the minimum lethal concentration for goldfish within one month.

In another series of experiments, one of us (W. J. D.) found that of two goldfish in 6000 c.c. water containing 10 c.c. $N/10$ lead nitrate (Pb 1 in 60,000), the fluid being changed on alternate days, one fish died on the tenth day and showed before death evidence of respiratory distress; post-mortem, its gill filaments were streaked with white lines of adherent precipitated protein which, on being placed in ammonium sulphide, was at once blackened (Fig. 1). Microscopical sections of the gills showed the black lead sulphide covering the surfaces of the gill filaments and lodged between their pectinations. Obviously, this fish died from the interference with the respiratory function of the gills.

Using solutions of 1 in 100,000 Pb, both as acetate and as nitrate, and with the fish in globes containing 1000 c.c. fluid, one found they

survived 17 days whether the fluid was changed or not; in fact the control fish in water not renewed but aerated daily died on the 14th day of the test, while the leaded fish survived this period of immersion in a very turbid fluid and when, after the 17th day, it was supplied with fresh "leaded" water every second day, it survived for four months and died only thirty-five days after being transferred to Pb 1 in 50,000. On filtering some of the first fluid, it was found to be quite free of dissolved lead which no doubt had become adsorbed and precipitated by the organic excreta of the fish. We did not find that renewal of the poisonous solution accelerated its toxicity; thus, one fish lived four

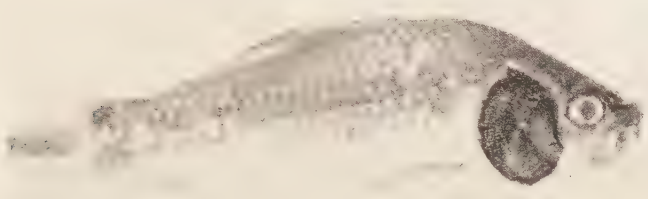


Fig. 1. Goldfish (nat. size) with operculum removed to show white precipitate of lead albuminate on gill filaments. The fish lived in 1 of Pb in 60,000 water for ten days.

months in Pb 1 in 100,000 and another two months in Pb 1 in 60,000; the former died 26 days after being transferred to Pb 1 in 50,000. We cannot accept the suggestion that the actual quantity of lead available determines the toxicity since our experiments with 1000 c.c. and 6000 c.c. of fluid negative this. We consider that there is a definite concentration of lead ion in water—about Pb 1 in 60,000—in which goldfish will survive for some weeks; below this concentration they may survive for some months; but, above it, they succumb very soon owing to interference with the respiratory function of the gills. When kept in concentrations of 1 in 200,000, there results a marked decline in weight which is comparable with the effects of slow absorption of lead in other animals.

Conclusions.

(1) No evidence has been obtained to show that traces of ionic lead in sea water retard the metamorphoses of plaice embryos.

(2) Lead in colloidal form does not kill young plaice in strengths of 1 in 250,000, but definitely retards their growth while they are in the

lead medium; the retardation seems to persist along with a lowered vitality after they are removed to a normal medium but accidents have prevented a final decision on this point being reached.

(3) The minimum lethal concentration of lead ion for goldfish is about 1 in 60,000; this concentration may be survived for a month at least; fish in 1 in 60,000 or stronger solutions may, however, die in 10 days with evidence of respiratory distress and precipitated protein on the gill filaments. Renewal of the fluid has not in our experiments accelerated the toxicity.

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(*Received November 11th*, 1925.)

INFLUENCE OF LEAD AND THE METALLIC IONS OF COPPER, ZINC, THORIUM, BERYLLIUM AND THALLIUM ON THE GERMINATION OF FROGS' SPAWN AND ON THE GROWTH OF TADPOLES[1]

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(With 1 Text-figure.)

The power of small doses of lead salts frequently administered to produce foetal death and abortion has been recognised only within recent times when lead compounds have become increasingly useful in industry. The mechanism of the abortifacient action has at various times been ascribed to spasmodic contraction of the uterus or its blood vessels, to placental haemorrhages and to direct toxic action on the embryo. Paul noted in 1860 that lead poisoning in either parent might result in abortions and that the mortality of infants born of lead-poisoned parents was unusually high; thus, of 141 mothers working in lead, 64 per cent. either aborted or had still-born infants while 25 per cent. of the live infants succumbed before the age of three; again, of 32 pregnancies by lead-working men, 12 resulted in abortions or still-births while only 2 of the live children survived the 3rd year (12). Verhaeghe quotes figures from 90 women workers in lead which show that 22 per cent. of their children were born dead and 19 per cent. of those born alive died before the age of two (14). Dr G. Reid compiled statistics which had similar import (13). Lewin (8) concluded that lead had deleterious actions on the germ cells of both males and females but Legge and Goadby (7) regard the case for poisoning of the male germ cell as unproved.

Experimental work on animals has materially clarified the situation. Oliver found that if hens' eggs were painted with lead nitrate solution they failed to hatch and the embryos reached only a limited stage of development, whereas control eggs painted with lime hatched (11). To

[1] This investigation was undertaken on behalf of the Liverpool Cancer Research Committee. Director: Prof. W. Blair Bell of the University of Liverpool.

what extent this experiment may have owed its success to checking of ventilation is difficult to say. More recently Cole and Bachhuber showed by experimental feeding of lead acetate to male rabbits and fowls that the offspring was smaller and of lower vitality (5). Almost simultaneously Weller (15, 16) demonstrated on guinea-pigs that the "leaded" males were either rendered sterile or, if not sterile, their offspring by healthy females was 18–20 per cent. below normal weight while "leaded" females had by a healthy male offspring about 15 per cent. below normal weight. Weller found that retardation of growth of the offspring persisted after birth—a feature noted by Oliver and Chyzer (4) in the families of Hungarian potters whose few living children grew slowly and were of short stature. Weller also had evidence that recovery of the power of producing normal offspring occurred in the adults after lead administration ceased. He detected no marked changes in the testes of the males, except in some cases rendered sterile in which there was complete aspermatogenesis with atrophy of the germinal epithelium. Morgan (9) has observed that sheep grazing on "leaded" land often abort and their lambs, if born alive, are undersized, delicate and rarely survive many weeks, but the parents, if transferred to wholesome pastures, will bear healthy lambs. Morgan's observations were made in Welsh lead-mining areas and he quotes the further relevant facts that fish apparently do not thrive in "leaded" streams, and rivers, formerly noted as spawning grounds of salmon and trout, are now devoid of fish—observations confirmed in part by Miss Carpenter (3); that dogs and cats fail to breed in the lead area but survive, although puppies and kittens succumb; that young ponies are very susceptible to lead; and that a mare—which remained unaffected—gave birth in 12 years to 5 foals which died from plumbism between the ages of two and five years.

From the above summary certain definite conclusions may be reached, namely, that lead is distinctly more toxic to germ cells and embryonic tissues than it is to adult tissues, and causes in the female either abortion or an undersized offspring; that it can affect both the maternal and the male germ cell; that its inhibitory effects on the growth and virility of the offspring tend to persist; but that surviving offspring become less susceptible to lead poisoning as age increases; and, finally, that the deleterious effects do not persist through births subsequent to protection of the parent from further lead intoxication.

This last point indicates that the seat of action of the lead in the mammal can hardly be on the unmatured ovum in the ovarian tissues, since then the effect would be more permanent. On the evidence it

seems more likely that the toxic effect is exerted on the ovum or developing embryo in the uterine cavity.

In the course of investigations on the toxic action of lead compounds on tissues, particularly on embryonic cells and malignant neoplasms, it was deemed advisable to ascertain whether lead salts produced any deleterious effects on amphibian spawn and upon the growth of tadpoles. The tests can be divided into three series: those in which the spawn was actually deposited in lead solutions, those in which spawn deposited in fresh water was divided up, a control kept, and the rest transferred to various lead solutions, and those in which tadpoles hatched in fresh water were transferred to lead solutions.

A brief account of the development of the frog's egg will suffice to illustrate the points noted in the experiments: Fertilisation occurs outside the body by the sperm shed over them. The ovum exhibits an upper portion deeply pigmented and a lower devoid of pigment but containing yolk; around the cell is the vitelline membrane and externally the jelly-like covering. Following the primary divisions resulting in a ball of epiblastic cells almost covering the yolk, the archenteron forms by a split at the junction of epiblast and hypoblast and a modified "gastrula" stage is reached—the embryo is now ovoid in shape (about 7th day). Later, as the notochord and nerve tube are developed, the embryo becomes flattened from side to side and appears reniform in shape (about 10th day). Embryonic development lasts about 14 days, during which the nutrition is lecithal and derived from the yolk. Just about the time of hatching the embryo is boat-shaped and exhibits eye-pits, external gills and the "sucker." After emerging, the young tadpole is herbivorous. The next stage is that of the internal gill period which commences about the 4th week and the limbs begin to show about the 8th week. By the end of the 2nd month the lungs come into use and the gills degenerate and about 3 weeks later the final metamorphosis into the frog takes place.

I. Experiments in which Spawning took Place into Lead Solutions.

In February and early March there were placed in sinks, each containing 2000 c.c. of increasing concentrations of lead acetate or lead nitrate, one male and three female frogs, the latter apparently ripe for spawning. Pond weeds were placed in the sinks and the solutions were changed twice weekly.

The following are the essential features of one such experiment:

Control. Two batches of eggs laid on 7th day, all spawn germinated, tadpoles $1\frac{1}{2}$ cm. long in 3 weeks; all four frogs survived.

Lead nitrate $N/20{,}000$. Two batches of eggs 5th and 7th days, a proportion of each batch germinated, eggs which germinated were tadpoles 1 cm. long in 3 weeks; all four frogs survived.

Lead nitrate $N/10{,}000$. Two batches of eggs 5th and 7th day, eggs commenced germination but proceeded no further than reniform stage (*i.e.* late "gastrula" stage) or 10th day of development; one female frog died.

Lead nitrate $N/2000$. Two batches of eggs 9th day, eggs did not germinate; all four frogs survived.

Lead nitrate $N/1000$. Two batches of eggs 5th day, no germination of spawn; all frogs died.

Lead nitrate $N/100$. Two batches of eggs and two frogs dead on 3rd day, one batch spawn and two frogs dead on 5th day, no germination of spawn, which showed evidence of protein precipitation and was contracted, *i.e.* individual jelly masses about one-half size.

In another experiment no germination of spawn occurred in solutions of lead acetate stronger than $N/20{,}000$ and, in one instance, although the female frogs were apparently distended with ova, no spawn was laid by the frogs in strengths of lead nitrate above $N/10{,}000$, while that laid in $N/20{,}000$ lead nitrate again failed to germinate.

In these experiments the lead salts might exert a toxic action on the parents, the extruded ova or the spermatozoon. The adult frogs cannot survive an $N/100$ solution of lead salt for more than a fortnight and are generally killed in 4 to 8 days; they become extremely sluggish and exhibit evidences of protein precipitation in the skin and external and internal mucous membranes; some will survive $N/1000$ for a month, but the majority die before this period; in weaker strengths the adult frog seems able to survive without showing symptoms for several months.

In regard to the ova the experiments have shown that germination of the frog's egg is inhibited by concentrations of lead salts above $N/10{,}000$, while $N/20{,}000$ prevents a variable proportion of the eggs undergoing development, but in this experiment the retardation of growth in the young tadpole is not striking. The fact that germination becomes checked when the embryo is about 10 days old will be referred to later. From these experiments there is evidence that the ovum is more susceptible to attack by the lead than the spermatozoon. The latter is poured over the eggs as they are extruded and it therefore must have survived contact with the lead, otherwise the partial germination which occurred in $N/10{,}000$ would not have occurred.

II. Experiments with Frog Spawn.

Experiments with batches of spawn obtained both from the stock sink in the laboratory and also from outdoor ponds were carried out both in bulk with portions split from several batches of spawn and also in smaller receptacles with portions of a single batch of spawn. The solutions were changed twice weekly; they were aerated and pond weeds were kept in them.

The following is the protocol of an experiment performed on large masses of eggs which, when placed in the solutions, had commenced germination and were about 5 days old. The observations are those recorded on the 9th day after placing in the solutions, *i.e.* 14th day after germination.

Control. About 75 per cent. germinated, tadpoles from $\frac{1}{2}$ to 1 cm. long, actively moving.

Lead nitrate $N/100{,}000$. About 20 per cent. germinated, tadpoles $\frac{1}{2}$ cm. long, movement sluggish, remainder of eggs checked at reniform stage (*i.e.* 8th to 10th day).

Lead nitrate $N/20{,}000$. A few tadpoles only have emerged but are dead, of the remainder about 25 per cent. show no development from spherical form, 75 per cent. have ceased development at ovoid or reniform shape (8th to 10th day).

Lead nitrate $N/5000$. No development has occurred, slight film of protein precipitation on outside of spawn.

Lead nitrate $N/1000$. No development has occurred, protein precipitation on spawn.

In experiments on a small scale, uniformity in the results is difficult to attain; the controls themselves often show a low percentage of hatches, due either to injury during division or to non-fertilisation, hence it is impossible to do more than form an average estimate of the percentage of eggs which fail to germinate in the weaker lead solutions. Further, after hatching, the young tadpoles are very liable to injury when the solutions are changed and casualties frequently occurred which could not reasonably be assigned to the poisonous solution, since they occurred also in the controls.

Below is given a synopsis of a typical experiment:

Control. 80 per cent. tadpoles hatched, size after 1 month $2\frac{1}{2}$ cm.; remaining eggs spherical.

Lead nitrate $N/500{,}000$. 10 per cent. tadpoles hatched same day as controls, they were a little undersized (3–5 mm.) during ensuing 14 days, size after one month $2\frac{1}{2}$ cm.; remaining eggs reniform or spherical.

Lead nitrate $N/100{,}000$. 25 per cent. tadpoles hatched 4 days late, survivors after 1 month $1\frac{1}{2}$ cm.; remaining eggs spherical or reniform.

Lead nitrate $N/20{,}000$. 10 per cent. reach hatching stage 6 days late, single survivor after 1 month 1 cm. long; remaining eggs spherical or reniform.

Lead nitrate $N/5000$. No development, slight albuminous precipitate in spawn.

Lead nitrate $N/1000$. No development, albumin precipitated, spawn contracted.

One must conclude from this and similar tests that $N/500{,}000$ usually, but by no means certainly or uniformly, arrests the development of a proportion of the frog's eggs exposed to it, but that the surviving eggs germinate normally and the tadpoles make progress practically equal to those in the controls. $N/100{,}000$ and $N/50{,}000$ solutions very definitely and sometimes wholly check germination, only a few of the eggs survive the ovoid or reniform stage, hatching is delayed, while survivors are distinctly undersized. With $N/20{,}000$ solutions, 10 per cent. was the highest proportion of eggs which attained development near or at the hatching stage and usually the tadpole died before detaching itself from the spawn; the remainder of the eggs died in the gastrula stage. In $N/10{,}000$, only one egg developed to a tadpole; in this and higher concentrations, arrest of development in the gastrula stage or before it was uniform.

There arises the question of the penetration of the lead salt through the albuminous envelope; it might be assumed that the lead would form a surface precipitate of protein on the spawn and so check its ultimate penetration to the ovum. Such an assumption is untenable, since from experiments with both egg albumin and serum, we have found that the precipitate which lead salts form with proteins is soluble in excess of protein, in fact, one can demonstrate the diffusion of redissolved lead ions through a layer of serum by means of ammonium sulphide. Further evidence on this point will be published later.

The second point of interest noted was that arrest of development almost invariably resulted when the egg was of reniform shape which corresponds with the late gastrula stage of the embryo and it was noted that, if the embryo passed this stage, it generally progressed to hatching even if it then succumbed. At the late gastrula stage when these embryos died the hypoblast is being formed at the expense of the yolk cells which later form a heaped-up mass on the ventral wall of the archenteron to be finally absorbed a day or two after hatching, by which time the mouth has opened. In view of the suggestion of Blair Bell(1) that lead arrests growth by a chemical reaction between the lead ion and such a phosphatide as lecithin the fact that the yolk cells are rich in lecithin must be borne in mind. It may be that the lead succeeds in entering the embryo by combining with the lecithin in the yolk cells and then

arrests by its toxicity further development of the hypoblast or of the epiblast which is dependent on the yolk for its nutrition. Presumably, on this theory, the embryo would be most vulnerable if the lead reached it before the yolk cells were finally covered by epiblast, that is, just before the stage at which the embryo becomes ovoid or reniform. If, however, the lead does not reach the embryo until the yolk cells and yolk containing hypoblastic cells are completely protected by the epidermal layer of epiblast, it is reasonable to expect, if Blair Bell's theory that the lecithin is the medium through which lead arrests growth is true, that the embryo would probably survive and develop to the tadpole stage and this is, in fact, what has been observed. Then at the tadpole stage, when it comes into direct contact with the poisonous solution, it may succumb by absorption of lead through the external gills or mouth. At present, these must remain as tentative suggestions to account for the observed facts; further work may confirm or disprove them. Many of the embryos in different stages of the above experiments were removed, and examined microscopically but nothing abnormal was discovered. Unfortunately, we possess no satisfactory micro-chemical method of detecting traces of lead in the tissues; were such available the above suggestions could be readily submitted to test. After gross poisoning, lead may be demonstrated in animal tissues as the sulphide.

Comparative tests were done between a colloidal preparation of lead and a molecular solution both containing comparable percentages of lead. The lead sol contained 0·25 per cent. lead as metal and hydroxide and had particles no greater in size than 0·2 μ.

Started 11. iv. 24	16. iv. 24	22. iv. 24
Control	50 % reniform	50 % tadpoles 1 cm. long
Lead nitrate N/10,000 (0·001 % Pb)	50 % reniform	No further development
Lead nitrate N/1000 (0·01 % Pb)	Eggs larger	No development beyond reniform stage
Colloidal lead (0·0016 % Pb)	50 % reniform	No further development
Colloidal lead (0·0165 % Pb)	70 % reniform	No further development

This shows that colloidal lead can also check development in somewhat similar percentages to those of molecular lead and presumably exerts its action after reverting to the ionic state.

III. Experiments on Young Tadpoles.

These were carried out on tadpoles at various stages of their free swimming state; the water was changed twice weekly and they were kept supplied with food in the form of fresh pond weeds, chiefly *Lemna*. The animals, being herbivorous, it is necessary to note that *Lemna* did not appear to suffer injury during the short time it was exposed to the lead solution. The possibility that the slowing in growth was due to poisoning of the food plants is therefore remote.

The experiments need not be detailed, as the chief results have already been indicated. There is no doubt, as might be expected, that very young tadpoles with external gills succumb more readily to lead

Fig. 1. Retardation of growth in a tadpole (left) which survived 16 days in a partly colloidal lead iodide solution containing 1 in 100,000 Pb. The tadpole on the right is a control from the same batch.

than those with internal gills. Lead nitrate $N/500{,}000$ does not, as a rule, materially delay development but tadpoles in $N/100{,}000$ are about one-half the normal size at the end of 1 month. Lead nitrate $N/50{,}000$ generally proves toxic to young tadpoles (external gills) in from 7 to 10 days and those surviving for 10 days are usually less than half the size of control animals. One which survived in $N/20{,}000$ for 1 month was only one-third the size of the controls.

Three tadpoles survived in lead nitrate $N/500{,}000$ until the month of October and then showed only a mere external trace of development of the posterior limbs—an obvious retardation of development. It was found, however, that some of the same batch which had been kept in an open-air aquarium had progressed no further. The aquarium was well filled with sand, water-weeds and algae and the cause in both cases

is obscure. Microscopical sections were made but the tadpoles were structurally perfect for their apparent age.

Tests made with colloidal lead preparations showed that these also retarded growth of the tadpoles. Young tadpoles survived in a partly colloidal lead iodide solution containing 0·2 per cent. Pb in a 1 in 500 dilution (*i.e.* 1 in 100,000 Pb) for 12 to 16 days, but at the end of that period they were one-half the size of the controls (see Fig. 1). It will be noted that this result is quite comparable with that achieved by ionic lead in similar dilutions.

IV. Action of other Metallic Nitrates on Germination of Frog Spawn and Development of Tadpoles.

Three long series of experiments were performed on identical lines with the above to ascertain whether other metals possessed actions resembling those of lead. For various reasons the metals zinc, copper, beryllium, thorium and thallium were utilised for comparison with the lead series; all were used in the form of nitrates and in dilutions of "normal" solutions in tap water. Two series were done with dilutions of $N/500{,}000$, $N/100{,}000$, $N/20{,}000$, $N/5000$ and $N/1000$ and one series with dilutions of $N/500{,}000$, $N/100{,}000$, $N/50{,}000$ and $N/20{,}000$. The synopsis of the results will be sufficient to indicate the differences noted in their effects:

Zinc did not appear to check development of the ova and, even from eggs in $N/5000$ and $N/1000$, tadpoles emerged but died immediately. Few tadpoles survived long in $N/20{,}000$ and those which survived in $N/50{,}000$ for over 3 months were stunted in growth and showed no evidence of limb buds.

Thorium. Development to tadpole stage in $N/100{,}000$, $N/50{,}000$ and $N/20{,}000$ was in the ratio of 50 per cent., 30 per cent. and 25 per cent. respectively, and $N/5000$ and $N/1000$ definitely arrested germination of the ovum. Tadpoles which survived $N/100{,}000$ and $N/20{,}000$ for 1 month were normal in size.

Beryllium does not interfere with development with certainty until a strength of $N/5000$ to $N/1000$ is reached; tadpoles may survive in $N/50{,}000$ and $N/20{,}000$ for 1 month and grow well; three survivals in $N/100{,}000$ for over 3 months developed hind limbs and were somewhat in advance of the controls.

Copper. In $N/100{,}000$ and $N/50{,}000$ and even $N/20{,}000$ development of the ovum as a rule proceeds normally until just before the tadpole emerges and is checked at this point. Even in $N/500{,}000$ few tadpoles

survive many days after leaving the spawn; a few survivors in this strength were three-fifths the normal size at the end of 1 month.

Thallium. $N/5000$ and $N/1000$ did not appear to delay development, but even in $N/500{,}000$ tadpoles on emergence are killed.

Conclusions.

Lead Salts. (1) Adult frogs are killed by $N/1000$ (Pb 1 in 10,000) lead solutions but usually survive weaker strengths. (2) There is evidence that lead $N/10{,}000$ (Pb 1 in 100,000) is rather more toxic to the ovum than to the free spermatozoon, but duration of exposure may have been a determining factor. (3) Lead salts $N/100{,}000$ to $N/20{,}000$ (Pb 1 in 1,000,000 to 1 in 200,000) can partly or wholly inhibit germination of frogs' eggs or they delay hatching. (4) Inhibition of germination occurs usually when the eggs are about the 10th day of development. (5) Lead salts $N/100{,}000$ (Pb 1 in 1,000,000) retard the growth of tadpoles which are one-half normal size at the end of 1 month, $N/50{,}000$ (Pb 1 in 500,000) generally kills in about 10 days, the tadpoles being about half normal size, while only rarely is $N/20{,}000$ (Pb 1 in 200,000) survived by young tadpoles, and these are always undersized. (6) Colloidal lead preparations in similar concentrations of Pb possess comparable actions.

Thorium Salts checked germination of the ova somewhat less than lead but did not, apparently, retard growth of tadpoles.

Zinc Salts in similar strengths did not check development of the ovum but were fatal to or delayed growth of the free-swimming tadpole.

Copper Salts did not arrest development of the ovum but were very fatal to the tadpole on emergence. Very weak solutions retarded growth.

Thallium Salts did not delay development but were toxic to the emerged tadpoles.

Beryllium Salts caused little developmental check and no marked delay in growth of tadpoles.

Summary.

(1) In these experiments lead salts proved to have a higher inhibitory influence on the germination of the frog's ovum than the salts of other metals tested and they also proved capable in weaker solutions of retarding the growth of tadpoles without causing their early death. Of the other metals tested thorium was active in checking germination; the others much less so. Zinc, copper and thallium were more toxic to the emerged tadpoles than lead. Beryllium was relatively inert. Hanzlick and Presho's observations on pigeons fed with pure metals led them to conclude that,

as regards the power of reducing body weight, the action of other metals was much less than that of lead, which they regard as having a specific toxicity (6). The present writers do not consider that these effects are "specific" or peculiar to lead, but they are relatively more marked with its salts. Locke's (9) experiments with tadpoles showed that lead was less directly toxic to them than zinc and much less than copper; this we have confirmed, but our experiments suggest that the "oligodynamic" action of lead decreases as the embryo ages.

(2) These experiments suggest that lead is more toxic to germinating tissues than the other metals tested and afford an explanation of its abortifacient action on the basis that it is much more toxic to the embryo than to the adult tissues of the parent. Subsequent experiments by Blair Bell, Hendry and Annett on pregnant rabbits have shown that after injections of colloidal lead there occurs a coagulation necrosis in the ectodermal tubules (trophoblast) of the foetal placenta; this rapidly spreads throughout the foetal ectoderm and into the adjacent superficial tissues of the maternal placenta so that the necrotic foetal placenta is soon cast off. They conclude that lead has a selective affinity for the chorion epithelium and that abortion can be produced by the action of lead on the foetal ectoderm (trophoblast) without the maternal organism being in any way affected.

On the findings of the present experiments these observers tried thorium as a substitute for lead but found it had no specific or toxic action on the chorion epithelium and that the abortifacient action of copper and thallium was uncertain and due to haemorrhage in the maternal tissues of the uterus (2).

(3) It has also been possible to confirm by these experiments some of the essential features recorded in the literature, *e.g.* (*a*) the toxicity of lead towards the female germ cell as higher than that to the male cell as was indicated in Weller's (15) experiments; (*b*) the greater susceptibility of very young animals to lead and progressive tolerance as the animal ages; and (*c*) the retardation of growth in the tadpole is comparable with the undersized offspring delivered from the mammal and the retardation of growth noted after birth by Weller (16).

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(*Received November 10th*, 1925.)

NOTES ON THE ENGLISH AND AMERICAN RACES OF THE GREENHOUSE WHITE-FLY (*TRIALEURODES VAPORARIORUM*)

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(With 5 Text-figures.)

The present paper represents a short account of somewhat scattered work on several aspects of the biology of the common greenhouse white-fly, *Trialeurodes vaporariorum*. It may be recalled that Morrill ('03) reported that in America the unfertilised eggs of this species give rise always to males. This was confirmed by Stoll and Shull ('19) as well as myself ('20). The breeding experiments of Hargreaves ('14) and Williams ('17) showed, however, that in England unfertilised eggs generally gave rise only to females. In one restricted locality only did Williams find specimens that seemed to furnish exceptions to this rule (see Table). Generally speaking, therefore, we may conclude that there are two races which differ in their reproductive processes, and based chiefly on the work just mentioned these races have been called the American and English race respectively.

In 1918, while working on the cytology of the American race I made some attempts to obtain material of the English race as well. Unfortunately, the stringent regulations of the United States government precluded the shipment of living specimens of the insect. However, through the kindness of Mr C. B. Williams and Mr E. J. Collins, some material fixed at my direction was shipped to me from Merton, Surrey, in the summer of 1920. A cursory examination of this material did not appear promising, and since conditions made it impossible to determine by breeding whether the stock from which the material was derived really belonged to the English race, I hesitated to begin the cytological study with the painstaking labour that it involved.

In 1922 Lloyd published some notes on the biology of *Trialeurodes vaporariorum*. I was surprised to find his results indicating that in the Lea Valley the reproductive behaviour of the insect is like that of the American race. This was indicated not only by sex-ratios in Lloyd's

random collections but also by actual breeding experiments. Virgin females gave rise to male individuals only.

My own efforts to obtain suitable, fixed material of the English race continued to be unsuccessful. My correspondence with English workers in this connection seemed to indicate, on the other hand, that Lloyd's findings might be duplicated in other localities, especially near London. Since Williams and Hargreaves had reached their conclusions in part on material from the general region, I became eager to investigate for myself the status of the two races in question. Opportunity to do this presented itself in 1924, when I was able to spend part of the summer in England.

Sex-Ratios in Random Collections.

As Williams showed, the fact that unfertilised eggs of the English race give rise to females is reflected in sex-ratios of random collections. Of some 500 specimens collected in various localities only 3 per cent. were males (this does not include the exceptional occurrence already mentioned). In contrast with this, when only males arise through parthenogenesis, there is a marked rise in the number of males in random collections. Thus collections in America usually show more than 30 per cent. males and often over 50 per cent., while Lloyd's collections showed about 50 per cent. of males. Since the time of my stay in England was limited and my primary purpose was to obtain material for a cytological study of the English race, I decided to make random collections and breed insects only from localities in which few or no males were present. If all virgin females bred from stock obtained in this manner produced only daughters, it would be fair to assume that the stock represented the English race.

For obvious reasons I made collections first of all in the localities which had furnished Hargreaves and Williams with their material. To make an easy comparison possible, the results of random collections reported by these two workers have been placed in columns parallel with mine. Additional collections are also given. At Berkhampstead, Kew Gardens, Harpenden, Luton, Merton and Wisley, collections were made from two or more different greenhouses which in some cases were some distance apart. Such collections were kept in separate vials until it was possible to examine the specimens and count the two sexes, but except in the case of Berkhampstead no significant variations were found in the various greenhouses of any one locality. With the exception noted therefore all the counts from one of the mentioned localities were lumped and are given as a single item.

Table.

Locality	Collector	Date	Females	Males	Collector	Date	Females	Males
Liverpool (Botanic Gardens)	Schrader	June 28, 1924	213	177	Williams	Feb. 28, 1911	46	1
Wisley, Surrey (Roy. Hort. Soc.)	,,	June 20, 1924	649	629	,,	Aug. 27, 1915	43	0
S. Kensington (Chelsea Phys. Gardens)	,,	June 17, 1924	185	216	Hargreaves	1914	Hundreds	0
Merton, Surrey (John Innes Hort. Inst.)	,,	June 23, 1924	658	698	Williams	Feb. 28, 1911	46	1
					,,	Mar. 22, 1912	6	1
					,,	Feb. 19, 1914	47	3
					,,	May 27, 1914	57	7*
					,,	Sept. 26, 1914	75	0
					,,	Feb. 11, 1916	137	0
Merton, Surrey (John Innes Hort. Inst.). In one restricted locality	Williams	July, 1915	229	98				
Lea Valley	Lloyd	July, 1920	144	161				
,,	,,	Oct., 1920	63	55				
Harpenden, Herts.	Schrader	June 16, 1924	87	79				
Luton, Bedford	,,	June 18, 1924	150	148				
Wheathamstead, Herts.	,,	June 19, 1924	174	167				
Kew Gardens	,,	June 21, 1924	202	259				
,,	,,	June 24, 1924	372	506				
Newcastle	Richardson	July, 1924	212	154				
Berkhampstead, Herts.								
Locality *a*	Schrader	June 22, 1924	554	245				
b	,,	June 27, 1924	86	95				
c	,,	June 27, 1924	25	10				
d	,,	June 27, 1924	144	74				
e	,,	June 27, 1924	235	58				
e	,,	June 30, 1924	92	28				

* Does not represent a truly random collection, since Williams was especially looking for males on this day.

Examination of the table will make it apparent at once that the great preponderance of females noted by Hargreaves and Williams obtains no longer, either in the localites from which they obtained their material or in several others. In fact, the sex-ratios are similar to those obtained by Lloyd in the Lea Valley and by several other workers in America. To conclude that the American race has supplanted the English race in all the places mentioned is, however, not justifiable on such evidence, for it is perfectly possible that individuals belonging to the English race are still present even in localities where the collections showed the greatest proportion of males. Only through extensive breeding experiments can this point be satisfactorily settled.

Breeding Experiments.

It will be seen that the lowest percentage of males was encountered at Locality *e* in Berkhampstead. This, then, was regarded as the most promising stock from which material of the English race might be obtained. Accordingly, virgin females of this stock were isolated on young tomato plants, and 21 of such isolated females laid eggs which developed and reached maturity. The broods ranged from 3 to 22 in number, most of them being small. The total number of offspring was 202, and all of them were males.

Despite the sex-ratio in the random collections, this stock thus probably belonged to the American race. Even more extensive breeding would be necessary to absolutely settle this point, and the same may be said of Lloyd's data in support of his contention that the Lea Valley strain agrees with the American race in reproductive behaviour. Lloyd bred only 5 virgin females, although the total number of offspring obtained was greater than mine. In both our experiments it is possible that some females of the English race were present, but happened not to be chosen for breeding. Finally, it is quite possible that interbreeding of the two races has occurred and that the parthenogenetic behaviour of the American race is dominant to that of the English race.

It may be said here that the work of Thomsen, whose preliminary note appeared recently, may throw light on this aspect of the question.

Cytology.

The only material possibly from the English race and available for cytological study was that sent to me from Merton in 1920. As my efforts to obtain authentic material of this race had proved unavailing, a re-examination of this shipment was made in 1922. The results were

inconclusive, and I therefore hesitated to publish them at that time. Since Thomsen's note ('25) indicates that he has been able to elucidate the points that have troubled me, only the shortest account of my work will be given here.

(*a*) There were 12 adults in the Merton material, and all of them were females. They were sectioned and three figures of the maturation divisions

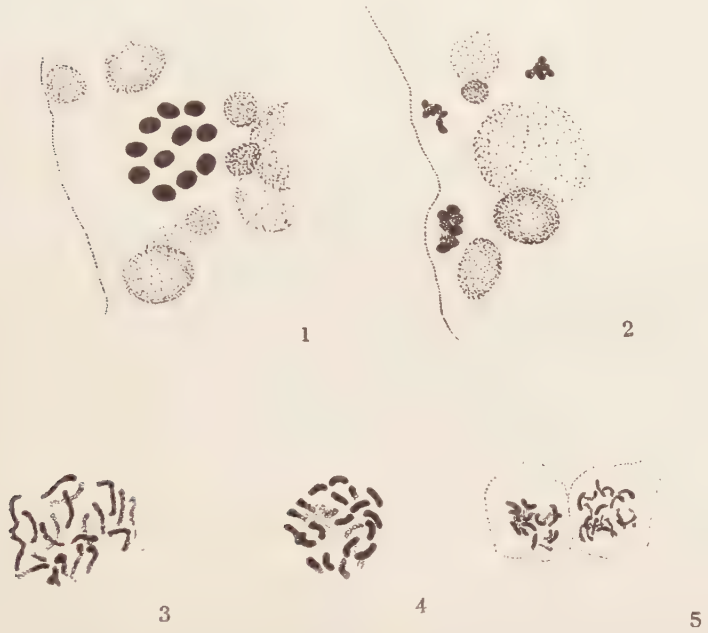

Fig. 1. Metaphase of first maturation division in the egg showing 11 tetrads.
Fig. 2. Section of egg showing first and second polar bodies at periphery and pronucleus somewhat deeper in egg.
Figs. 3 and 4. Chromosomes in nuclei during early cleavage stages. Close to 22 chromosomes present.
Fig. 5. Chromosomes in cells of the germ streak stage. Definitely more than 11 chromosomes present.

were found. One of these figures was in two sections, one of which was lost in restaining. The remaining section shows 7 complete tetrads and part of an 8th, and these tetrads are not yet fully condensed. The other figures show 11 tetrads exactly like those observed in the American race (Fig. 1).

(*b*) One figure in the stage immediately following the maturation divisions was found. It shows two polar bodies and the female pro-

nucleus (Fig. 2). Again, as in the American race, two maturation divisions are apparently completed here.

(*c*) Among the developing eggs, the majority showed no chromosome plates from which counts or even estimates could be made. Among those where this was possible, 4 were in the cleavage stage, 2 in the late blastula stage, and 10 in varying stages of the germ streak formation. In all cases the number of chromosomes involved was close to 22 or at least definitely greater than 11 (Figs. 3, 4 and 5).

Counting all embryos, a total of 16 individuals in which the number of chromosomes was close to 22 or at least definitely more than 11 was found. Only 1 embryo showed figures in which 11 chromosomes may have been present, but these may just as well have contained 22. If, therefore, only two classes of individuals are present in the English race just as in the American race, that is, females with 22 chromosomes and males with 11 chromosomes (and Thomsen's results seem to show that this is indeed the case), it may be concluded that the embryos in question represent 16 females. Together with the 12 adult females in the material, this makes a total of 28 females as compared with 1 or no male. It is therefore likely that this shipment from Merton represents the English race.

If that is the case, my cytological findings permit the following conclusions:

Pseudo-reduction and two maturation divisions occur just as in the eggs of the American race.

The chromosomes are doubled between the time of the formation of the second polar body and the early cleavage divisions.

My material does not allow me to decide whether this doubling occurs by a refusing of the second polar body with the pronucleus or some process in the cleavage nuclei. Nor am I able to settle the question of the origin of the few males that occur in the English race. As far as they go, my results seem to support those of Thomsen, who will no doubt be able to clear up the questions that I have been puzzled about for so long.

Conclusions.

It was my policy to elucidate as much information as possible from gardeners and other practically interested persons about the white-fly question. It was of some interest to find that a summing up of their opinions would indicate that in the localities investigated by me the white-fly plague has reached its present serious proportions only within

the last 15 years. Previous to that, white-flies seem to have been known certainly, but were not regarded as a specially dangerous pest.

The one point definitely indicated by my data is that the American race is spreading rapidly to all places where it was formerly unknown. The alternative explanation advanced by Williams ('17) that "some conditions have caused our English race to pair more frequently and polygamously which would result in an immediate increase in the number of males towards a position of stability at equality of sexes" can hardly be held any longer.

The correlation between the spreading of the American race and the apparent increase in the number of white-flies seems to show one or both of two things: The American race reproduces at a greater rate or it is more resistant to detrimental environmental factors. Such a conclusion is not taking into consideration the possibility of a change in environment (as for instance a tremendous increase in the raising of tomatoes) or the fact that interbreeding between the English and American races may have produced a more vigorous strain. It is to be hoped that Thomsen's more detailed paper may clear up the latter possibility.

Thomsen's work indicates that *Trialeurodes vaporariorum* is to be found in Denmark. Although it has therefore reached the continent, it apparently has not yet become a pest, at least in Germany, as my search for it there in 1924 would seem to show. No specimen of the insect could be found in one or two greenhouses searched at Magdeburg and Berlin. But further north, nearer the coast, where it may be expected to appear first, my searches were no more successful. At Lübeck several greenhouses were inspected at two different localities (two of them containing nothing but tomato plants). At Kiel I searched the greenhouses of the Botanical Garden of the University, and also the greenhouses at two places in the suburbs of the city (at both of which tomatoes were extensively cultivated). No trace of *Trialeurodes vaporariorum* was found anywhere, although I was informed at the University gardens that insects fitting my description of the white-fly had been seen on cabbage in the previous autumn. The chances are that even if these were white-flies they were not *Trialeurodes vaporariorum*.

I take this occasion to express my indebtedness to the persons who have aided me in my work, notably Messrs C. B. Williams, E. J. Collins, R. E. Richardson, Professor J. W. H. Harrison, and my wife, Sally Hughes-Schrader. My especial thanks are due to Dr A. D. Imms, of the Rothamsted Experimental Station, who furnished me with working

quarters and a greenhouse during my stay in England. An appropriation from the American Association for the Advancement of Science to aid me in defraying my expenses is also gratefully acknowledged.

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(*Received November 9th*, 1925.)

ON THE EARLY STAGES OF SOME WEEVILS (CURCULIONIDAE)

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Part I.

(With 24 Text-figures.)

CONTENTS.

		PAGE
Introduction		197
1. *Phyllobius urticae* Deg.	(*a*) Biology	198
„ „	(*b*) Mature larva	199
„ „	(*c*) Pupa	209
2. *Phyllobius pyri* L.	(*a*) Host-plants of larva	210
„ „	(*b*) Mature larva	210
„ „	(*c*) Pupa	213
3. *Polydrusus cervinus* L.	(*a*) Biology	214
„ „	(*b*) Mature larva	216
„ „	(*c*) Pupa	217

Introduction.

The larvae of most weevils of the family Curculionidae bear, superficially at least, such a strong resemblance to one another that many of those who have set out to describe them have left us with descriptions inadequate to the purpose of separating the different species. In recent years, however, some progress has been made by authors such as Grandi and Urban, who have made a more precise study of morphological details or have compared a larger series of different larvae. Others such as Verhoeff (17) and Cotton (5) have even attempted to give us generic characters for a limited number of larvae, but though the work that has been done in this sphere is of undoubted value, there are as yet too many gaps in our knowledge of the larvae of even the commonest species to justify this being done on any but the most limited scale.

The present work was undertaken in the hope of making a contribution to the filling up of some of these gaps, especially amongst those

weevils which spend their larval life in the soil. The difficulty of obtaining ova from the beetles in pots and of rearing these delicate larvae through all their stages has been found so considerable that, while not entirely abandoning the obvious method, resort has been made to collecting larvae in the field, fixing upon characters by which they may be identified and finally rearing a sufficient number to the adult stage to establish with certainty the identity of the larvae.

Phyllobius urticae has been used here as a type merely for convenience and not as implying that it has any claim to that position on grounds of phylogeny.

The characters found most useful in differentiation of these larvae have been the spiracles (which sometimes differ as between the thorax and abdomen), the cuticular armament, the antennae and the presence or absence of ocelli.

In the early stages of the work the writer had the advantage of a grant from the Ministry of Agriculture and Fisheries, to whom he desires to express his thanks. Acknowledgment must also be made of the kindness of Mr J. C. F. Fryer, director of the Ministry's laboratory of plant pathology, for providing certain named larvae at the outset—a valuable contribution at that stage—and of that of Mr G. Fox-Wilson, of the Royal Horticultural Society's laboratory, for most valuable and interesting material of *Otiorrhynchus*, to be dealt with in a future part.

Finally to Dr D. Keilin, of this laboratory, thanks must be given for his ready counsel in all matters relating to morphology and physiology, subjects in which his experience is of the greatest value.

1. **Phyllobius urticae** Degeer.

(*a*) *Biology.*

Little is known of the life-history of this species. Urban obtained eggs on the roots of a nettle and reared the beetles through to maturity in his garden (14). This is, as would be expected from the common occurrence of the adults there, the usual host-plant, but Fryer bred the weevils also from larvae found at the roots of strawberry plants (6), and probably other food-plants may be made use of on occasion. I have found them abundantly at the roots of nettles near Cambridge on several occasions in the early spring. Pupation takes place in an earthen cell a little beneath the surface of the soil from the middle to the end of March, and the adult weevils emerge from the pupal case some three to four weeks afterwards. Presumably, the eggs are laid in May and June and the

larvae undergo the major part of their development before hibernating in that state.

A larva has been found attacked by a fungus-parasite, which Mr A. T. Speare, of the United States Department of Agriculture, has kindly identified as belonging to the genus *Beauveria*, a genus also found attacking the aerial larva of *Apion apricans* on clover.

(*b*) *Mature larva.*

Length extended 9–10 mm., breadth 4 mm.

Like most other larvae of this family, it is curved ventrally, legless, of a creamy white colour and, except the well-chitinised head-capsule and slightly hardened pronotum, is soft and unprotected by chitinous plates. The body tapers gradually from the metathorax, the broadest

Fig. 1. *Phyllobius urticae* Deg. Full-grown larva. ×12.

segment, to the 9th abdominal segment. The tergites of the abdominal segments 1 to 5 or 6 are furnished with numerous minute brown cuticular spinulae (Fig. 2), which extend over the dorsal surface almost to the spiracles on either side, avoiding the tubercles on which the setae stand. Under a high magnification still more minute colourless spinulae may be found on the thoracic and other tergites and especially in that region of the 10th abdominal segment surrounding the anus. From the ventral surface they are almost entirely absent and on the pleurae, apart from the 10th, are only visible in a limited area on the 8th and 9th abdominal segments.

The *head capsule* (Fig. 3) is brownish yellow and, viewed from before, is almost round, though it is flattened anteriorly (below) for the attach-

ment of the appendages. Dorsally the pale epicranial suture extends medianly from the occipital region for rather more than half the length of the head and then branches, each branch running, almost at right angles to the other, to the base of each mandible (frontal sutures). The brown median frontal carina often found in weevil larvae is absent. The two epicranial plates, which are separated from one another by the epicranial suture, are rounded at the sides and terminate on the ventral side of the head. Here each forms one margin of the wide occipital foramen and is continued anteriorly beneath the maxillary cardo to the anterior margin of the head, where it forms the socket in which the large ventral condyle of the mandible articulates.

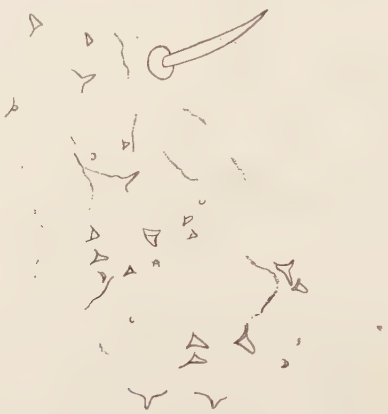
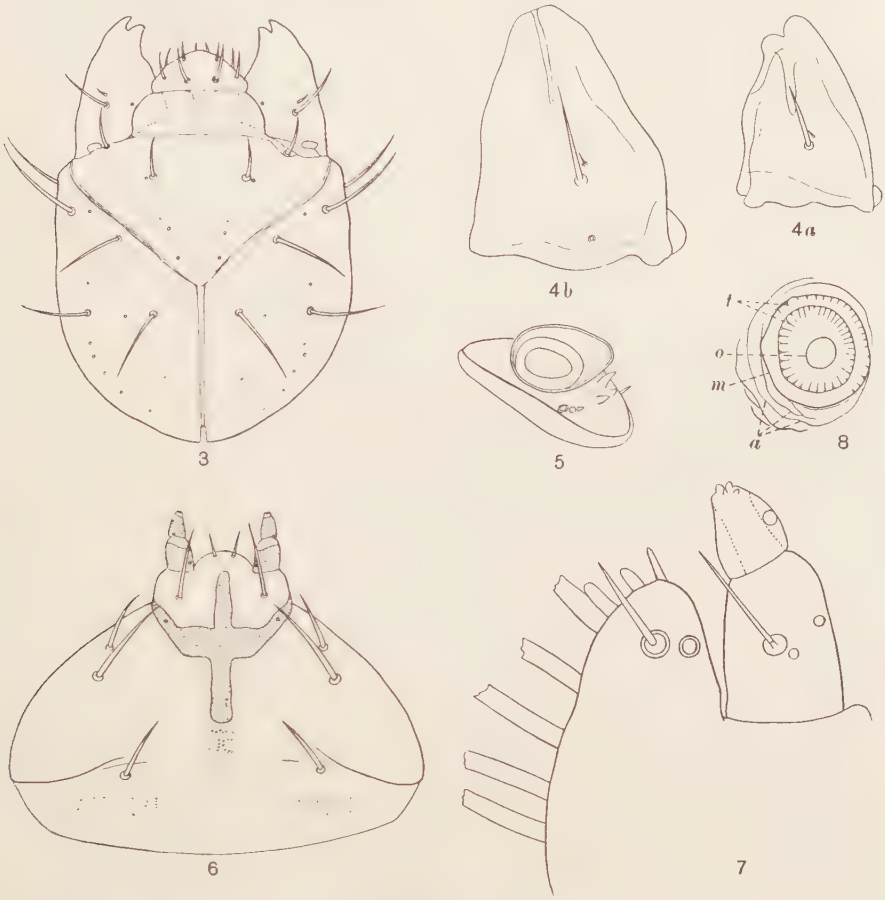


Fig. 2. *Phyllobius urticae* Deg. Cuticular spinulae on 2nd abdominal tergite. ×280 *circa*.

The *tentorium* is but little developed on the dorsal surface, only following the epicranial suture for a short distance from the occiput. Ventrally it forms a margin round the inner surface of the epicranial plates up to a point half-way between the occiput and the anterior margin of the head. The two sides of the tentorium coalesce at this point, forming an arch which supports the condyles articulating with the maxillary cardines. The anterior margin of this arch is somewhat excavated in the middle and is produced into two short processes for the attachment of muscles. From this arch the tentorium is continued forwards beneath the margin of each epicranial plate to the base of the mandible on either side, while across the anterior margin of the head a further apodeme, in the form of a transverse bar, extends from the inner angle of one epicranial plate to that of the other. Medianly this bar is a little produced anteriorly and is at that point firmly connected with the interior lining of the sub-

mentum. Here there are also, side by side, two brown spots, the function of which is unknown to me. (The tentorium of *P. pyri*, similar to that of the present species, is represented in Fig. 11.)



Phyllobius urticae Deg.

Fig. 3. Head of larva, dorsal surface. ×39 *circa.*
Fig. 4. Mandible (*a*) of immature larva, (*b*) of full-grown larva. Each ×65 *circa.*
Fig. 5. Antenna of larva. ×212 *circa.*
Fig. 6. Labium, showing chitinous process. ×65 *circa.*
Fig. 7. Maxillary mala and palp. ×212 *circa.*
Fig. 8. Thoracic spiracle. ×212 *circa. a.*, annulae; *m.*, margin of spiracle; *o.*, opening of spiracle; *t.*, trabeculae within the atrium.

Chaetotaxy of head. Each epicranial plate has two setae a little below the vertex, the four setae being almost in a transverse line. In addition there is a single seta on each side on the margin of each branch of the

frontal suture, a rather long seta anteriorly behind the mandible, two similar setae on the lateral margin and one ventrally, these four last being placed near the anterior angle of the head.

On the frons there is a single rather short seta almost at the anterior margin, on the inner side of each antenna, while behind these is a pair of longer setae, one on each side of the median line. Urban in a recent paper on *Otiorrhynchus sulcatus* (15), following Spessivtseff (11), has sought to establish specific characters in the arrangement of the microsetae and sensory pores of the larval head. The heads of a number of larvae of several species have been examined with this end in view, but the microsetae have been found to vary too much to justify the adoption of this method in the opinion of the present writer, though in certain cases it is not denied that clearly defined and constant characters of this kind may be found. Those found in a fairly representative head of the present species are indicated in Fig. 3.

The *mandibles* (Fig. 4) are yellow, pitchy at the apex, where a somewhat deep indentation separates the two apical teeth. These are usually much eroded and the indentation appears as a mere suture separating two rounded projections. Near the middle of each mandible there are two setae, one of moderate length, the other short, situated close together. Inner margin with a scarcely perceptible flange or tooth.

The rudimentary *antennae* (Fig. 5) are placed on the anterior margin of the frons (or epistome), at the base of the mandibles. Each consists of a basal plate, on which are situated a small sensory pore and seven sensory processes. One of these is much larger than the rest, hyaline, oviform, with the pointed end turned asymmetrically towards the lateral margin of the head. The remaining six processes are minute papillate structures, situated between the largest process and the lateral margin.

Ocelli absent. *Labrum* nearly semi-circular, slightly undulating and provided on the anterior margin with eight down-curving setae in pairs, in addition to a pair of shorter much-curved setae, situated one on either side of the median pair. All these arise on the dorsal surface. There is also a row of four longer setae near the base and a similar pair anterior to these, near the middle. The under surface is almost covered with minute cuticular scales. Medianly the epipharynx extends forward in two strongly chitinised arms, which arise beneath the clypeus, diverge a little, and almost reach the anterior margin of the labrum (see Fig. 20, of the same organ in *Polydrusus*). The area enclosed within the epipharyngeal arms is smooth, but bears three pairs of short peg-like setae. The first pair is placed close together near the anterior margin and is

followed by a second pair, wider apart, enclosing between them a pair of tripartite taste-buds. The third pair arise some distance within the arms of the epipharynx, rather nearer together than the preceding pair.

On either side of the epipharynx, covering the arms, but leaving bare the median area, there is a flap of membrane thickly covered with sharp spinous cuticular processes, pointing inwards. Probably these assist in directing food down the median area.

From the base of the labrum to the base of the clypeus a dark spatulate process of chitin extends between the epipharynx and the outer surface of the clypeus.

The *clypeus* is transverse, almost straight anteriorly, rounded at the sides and bears four short rudimentary setae in a transverse row at the base.

On the inferior surface, the sclerites of the *labium* (Fig. 6), with the exception of the terminal lobe, are pale and membranous. The basal portion, probably the *submentum*, is imperfectly separated from the mentum by a slight suture and in a fresh specimen may be said to form, roughly, an obtuse-angled triangle. A short seta is placed near each of the acute lateral angles. There is, however, a further area of membrane at the base of the submentum, which is folded beneath it but becomes visible in a prepared specimen. The *mentum* is divided medianly by the terminal lobe and its chitinous process into two parts, each of which is pale and membranous and bears two setae of moderate length in a line parallel to the terminal lobe. The *terminal lobe* is scutiform, the narrow basal part resting upon the submentum in the median line. It bears a pair of two-jointed *palps*, each of which is brownish yellow in colour and rests upon a pale palpiger. The basal segment is broader and somewhat longer than the apical segment. Several minute sensory pegs or cones are situated on the apex of each palp. In the median line and on either side of the terminal lobe there are yellowish brown stripes of stronger chitin, which are united together and continued in a basal stripe reaching to the submentum, the whole forming a trident-like structure of hardened chitin. The rest of the lobe, including the short rounded ligula between the palps, is pale and membranous. A rather long seta is placed near the base of each palp and two pairs of short straight setae, the one on the outer, the other on the inner surface of the ligula.

The *maxillary palps* are yellowish brown in colour and are each two-jointed. The basal segment is nearly twice as long as the terminal segment and considerably broader. It bears a short seta near the middle as well as two sensory pores. The terminal segment is bluntly pointed and has

a single sensory pore half-way between base and apex and some 7 or 8 minute sensory pegs at the apex. Each palp stands upon a white, thinly chitinised palpiger with two short, curved, setae at its base. The greater part of the *stipes* is yellowish brown and bears a rather long curved seta on the outer margin, midway between the palpiger and the base. The base is truncated and almost straight. On the inner margin of each maxilla there is a single unsegmented *mala*, which is flattened, rounded on its inner margin and runs to an obtusely pointed apex (Fig. 7). The inner margin shows a row of nine or ten stiff bristles, which project inwards across the entrance to the mouth, but arise from within the margin of the mala. On the ventral surface, near the apex, there is a single longer seta and behind it a small sensory pore. There is also a very short seta and sensory pore near the inner margin, almost in line with the base of the palpiger. The mala is strongly excavate at its base, on the side bordering the palpiger, as shown in Fig. 16.

The maxillary *cardo* articulates with the straight-ended stipes in a plane at right angles to that of the face of the stipes. It lies along the edge of the mentum and submentum and at its base articulates with the process already described as arising jointly from the tentorium and the inner margin of the epicranial plate. It is yellowish brown in colour and roughly triangular in shape, the apex of the triangle articulating with the tentorial process.

In Verhoeff's table of genera(17) *Phyllobius pyri* is taken as type of the genus, the principal, though not the only, characters used being the head-appendages. From the characters given for *Phyllobius* the present species differs in the shape of the terminal process of the antenna, which, instead of being elliptical as in *P. pyri*, is produced into a blunt point on the outer side. Other characters by which *P. pyri* may be distinguished from *P. urticae* will be found in the section dealing with the former.

Segmentation of the body. As in all coleopterous larvae which lack a strong chitinous cuticle, the body is divided into definite areas, subdividing the true segments (Fig. 1). These areas depend for their form on the attachment of the muscles beneath them.

The *pronotum* is slightly more corneous than the other segments and undivided, save for a slight indication of a praescutum behind the head. The *meso-* and *meta-nota* are each divided by a transverse suture into two areas, the anterior or praescutal fold and the posterior or scutellar fold (the scutum and scutellum are not differentiated). Of the *abdominal segments*, the 1st to 7th have three dorsal areas, the praescutum, scutum

and scutellum, separated by transverse sutures; the 8th is obscurely divided into two areas, while the 9th and 10th are undivided.

The *pleural* region is occupied by large setiferous tubercles, of which only one, the hypopleural tubercle, is present in the prothorax, two in the meso- and three in the metathorax. Each abdominal segment from the 1st to 8th has two, epipleural and hypopleural tubercles, respectively above and below the pleural suture, which is continuous from the 1st to the 9th segment of the abdomen. In the 9th segment the suture is interrupted by the presence of the single pleural tubercle, which arises at this point on either side.

Ventrally, each segment from the prothorax to the 8th abdominal segment is divided into two transverse folds, an anterior sternal fold and a posterior sternellar fold. The sternal fold occupies the greater part of the ventral surface of the segment, the sternellar being narrow and without setae. The 9th sternite is undivided. The 10th is divided into four divisions by the radiating sutures of the anus.

Chaetotaxy. On either side of the medio-dorsal line the pronotum bears 11 setae, longer or shorter, situated mostly a little above the spiracle. The prothoracic pleura has two rather long setae, borne on the hypopleural tubercle just behind the head. Ventral to it lies the round, projecting crural pad, which bears 6 setae, most of them long and tapering. On the prosternum, between the two crural pads, there is a pair of rather short straight setae, arranged transversely.

The mesonotum bears a pair of long setae dorsally on the anterior fold and a transverse row of five, three short and two long ones, on either side of the median line of the posterior fold. Three epipleural setae are placed on an elongate tubercle, situated obliquely behind the prothoracic spiracle, one of these setae, the most dorsal, being extremely short. The single hypopleural seta is placed on a tubercle adjoining the mesothoracic crural pad. The sternal setae have the same number and position as those of the prosternum. Dorsally and ventrally the metathorax has the setae arranged similarly to those of the mesothorax. The four pleural setae however are arranged differently, on three separate tubercles; the epipleural, two setae, one of them extremely small, placed side by side almost horizontally; the pleural seta intermediate; and the hypopleural seta on a tubercle adjoining the crural pad. In the abdominal segments 1 to 5 each tergite bears 8 setae on either side of the median line as follows: one on the dorsal surface of the anterior fold; two laterally, a longer and a shorter one, on the intermediate fold above the spiracle; five on the posterior fold, the median one short, the rest

alternately longer and shorter, in a transverse row. There are four pleural setae, two each, a longer and a shorter one, on the epipleural and hypopleural tubercles. The sternite has a transverse row of six stiff tapered setae.

The 6th and 7th abdominal segments vary from the rest in having only one short lateral seta above the stigma on the intermediate fold of the tergite, while the 8th tergite has a similar short lateral seta and only four setae in the posterior row on each side of the median line.

The 9th abdominal segment has only three dorsal setae on either side of the median line and on the single pleural tubercle a long tapered seta, with a rudimentary one a little dorsal to it. The sternite bears a row of four setae, arranged transversely.

The 10th abdominal segment has two small setae on each of the two lateral quarters posteriorly. In *Otiorrhynchus cribricollis* similar setae are considered by Grandi (7) to belong to the sternite.

The following table, after Grandi's method, gives a summary of the number of setae belonging to each segment:

Somite	Tergite	Pleurite	Sternite
1st thoracic	11+11	2+2	7+7
2nd ,,	6+ 6	*4+4	7+7
3rd ,,	6+ 6	4+4	7+7
1st–5th abdominal	8+ 8	4+4	3+3
6th–7th ,,	7+ 7	4+4	3+3
8th ,,	6+ 6	4+4	3+3
9th ,,	3+ 3	†2+2	2+2
10th ,,	—	—	2+2

* Sometimes a minute additional seta is present in the epipleura.

† Including a minute seta.

Spiracles. The functional spiracles of the thorax are situated in the postero-lateral part of the pronotum, there being no outward sign of the metathoracic spiracle. In each of the first eight abdominal segments they are situated laterally in the intermediate fold of the tergite, a little dorsal to the epipleural tubercle.

The external aspect of each spiracle (Fig. 8) is circular, the roof of the atrium or respiratory chamber being formed by a brownish membrane. In the centre of this membrane there is a small circular aperture (*o*, Fig. 8), which is the functional spiracular opening. Around the margin of the spiracular membrane there are variable numbers of colourless annulae, representing the peritreme, but there is otherwise no sharply defined margin to the spiracle.

Within the atrium are two concentric rows of short colourless processes or trabeculae, attached to the walls of the thoracic spiracle, while in the abdominal spiracle these are reduced to mere rudiments. The inner aspect of the atrium converges conically to a short trachea, at the end of which the closing apparatus with its two levers is placed. This short trachea, called by Steincke (12) "stigmengang" and by Alt (1) "stigmengrube," is particularly short in this species. It is referred to here as the "intermediate trachea," signifying that it is intermediate in position between the atrium of the spiracle and the closing apparatus.

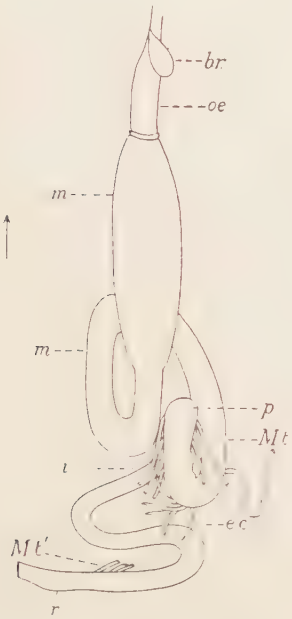


Fig. 9.

Fig. 10.

Phyllobius urticae Deg.

Fig. 9. Alimentary canal of larva (diagrammatic). *br.*, brain; *e.c.*, enteric caeca; *i.*, ileum; *m.*, mid-intestine; *M.t.*, Malpighian tubules, insertion; *M.t'.*, secondary attachment of Malpighian tubules; *oe.*, oesophagus; *p.*, pyloric thickening; *r.*, rectum.

Fig. 10. Pupa. ×12.

Alimentary canal (Fig. 9). The *oesophagus*, which is gradually widened from the pharynx, is short and straight and only extends a short distance into the prothorax. A slight constriction separates it from the *mesenteron* or stomach, which in a fully-fed larva is nearly tubular and of but little greater diameter than the oesophagus. In a larva which is still feeding, however, the anterior part of the stomach is distended with food immediately behind the oesophageal valve and is then considerably the widest

part of the alimentary tract. After a rather gradual constriction the mesenteron bends to the left and then, crossing beneath the anterior portion to the right side, is twice bent in a complete loop at the pyloric end. At this point the walls of the alimentary canal are greatly thickened and the six *Malpighian tubules* are given off from it. These, after extending forwards as far as the anterior margin of the stomach, return backwards to the posterior end of the body and are finally superficially reattached to the wall of the colon, posterior to the final bend of the hind intestine. Each tubule is unbranched and of great length. From the point of attachment of the Malpighian tubules the *hind intestine* proceeds backwards and, after making two more sharp bends, takes a straight course to the anus. The *rectum* is short and can only be recognised from the rest of the intestine with difficulty until it is cleared in oil, when the interruption of the longitudinal muscles renders it easily distinguishable. At the most posterior bend of the mesenteron, just before it takes the final pyloric bend, five moderately long and pointed *enteric caeca* are given off.

The dilated anterior portion of the alimentary canal of *Calandra sommeri*, figured by Burmeister and reproduced by Newport (10), is referred to by the latter as a "bag-shaped crop." He refers, however, to the valve by which the oesophagus opens into this organ and adds that it is "analogous probably to the first stomach of the larva of *Calosoma*." Such an explanation would tally with the arrangement found here and also with that found by Verhoeff in *Cionus solani* F. (16)[1]. The Malpighian tubules, or at least some of them, contain many scattered crystalline bodies in the lumen. These have been submitted to Dr Keilin, who says that they differ from the calcospherites found by him in the Malpighian tubules of *Acidia heraclei* (8). In appearance they are spherical or almost so, with an outer layer differentiated from the central core, which contains a central nucleus, with lines radiating from it to the circumference. They are brownish in colour and are evidently not composed of lime, as they are able to withstand the strong acetic acid used in fixatives such as Carnoy's solution. Similar bodies have also been found in *Otiorrhynchus sulcatus* and it seems likely that they will be found in other species. It is rash to guess at the composition of these spherites, but it is perhaps significant that Marchal (9), in his large paper on the presence of uric acid in invertebrates, gives a large number, including coleoptera, in the Malpighian tubules of which uric acid and urates have been found.

[1] Schultz has also found two compartments of the mesenteron in the larval *Anthonomus pomorum*. *Zool. Jahrb.* (*Abt. Syst.*) (1924), XLVIII, 248.

Central nervous system. The central nervous system extends from the head to the anterior part of the 4th abdominal segment. The ganglia, of which there are three thoracic and seven abdominal, are placed very close together, so that they appear, when viewed from the dorsal surface, almost as if fused together and to the suboesophageal ganglion of the head. When viewed from the side, however, they are seen to be distinct, the shortness of the two connectives on the dorsal surface, together with the overlapping of the individual ganglia, having caused the appearance of fusion. Of the thoracic ganglia, the metathoracic ganglion is considerably larger than either of the others or than the suboesophageal ganglion and thicker in front than behind. Of the abdominal ganglia, the 6th is considerably longer than any of those that precede it, but the 7th (which is doubtless a compound ganglion, belonging to three or four segments) has a mass more than twice that of the 6th. From each ganglion nerves extend to the segment to which it belongs, generally ventrally, though in some cases the nerves arise dorsally and proceed for some distance on the dorsal surface of succeeding ganglia.

(*c*) *Pupa.*

Length in curved position 6·5 to 7·0 mm. Width across prothorax about 4·5 mm.

Creamy white, the surface of the cuticle furnished with a close felt of minute pale spinulae. General form as shown in Fig. 10.

Head with five pairs of setae on the dorsal surface, each, except the finer pair of setae above the imaginal eyes, placed on a raised papilla as follows: one pair on the vertex; two setae a finer and a stouter one above the imaginal eye on each side; one pair between the eyes; one pair between the bases of the antennae.

Pronotum with an anterior row of six setae in a transverse row and behind these a single seta on each side; posteriorly on the scutellum six setae in a wide V-shaped formation, pointing anteriorly. All on raised tubercles, which are themselves furnished with cuticular spinulae.

Mesonotum with a row of four setae on the anterior margin. Behind these there is a crescentic tubercle on either side of the median line, each bearing four setae on separate papillae. Anterior and somewhat lateral to the tubercle there is a short stiff brown seta and near the insertion of the elytral sheath a fine seta, neither of these being upon tubercles.

Metanotum with an oval-shaped tubercle, which carries four setae, placed on separate papillae, on each side, similar to that on the mesonotum. Antero-laterally to this there is a short brown seta arising directly from the surface of the cuticle and at the anterior margin of the segment

there is a pair of fine setae placed one on either side of the median line. With the exception of these fine setae and the others mentioned above, all the thoracic setae stand on tubercles furnished with fine cuticular spinulae.

The leg-sheaths bear setae at the joint between femur and tibia, two on the first and one on each of the other legs, each on a short papilla.

Abdominal tergites each with a transverse row of short, stiff, tapered setae or spines borne separately on papillae, which lack the cuticular spinulae present on those of the thorax and the general surface of the cuticle.

Segments 1 to 7 each with five pairs of spines dorsally and a further two setae placed laterally on each side a little anterior to the dorsal row. On the pleurae of segments 3–7 there are two short, fine, pale setae, one dorsal, the other ventral.

Segment 8 has a group of three spines dorsally, arranged in a triangle and more laterally a group of two spines side by side, with a finer one anterior to the two others. The pleurae bear three short brownish setae on either side. Segment 9 has a pair of rather short, stout, incurved spines at the apex, each borne on a somewhat prominent tubercle, which also carries three more slender brown setae of differing length. In the pleural region there is a longer and a shorter seta on either side. Ventrally the segment has a single short pale seta on either side of the scutum.

2. **Phyllobius pyri** L.

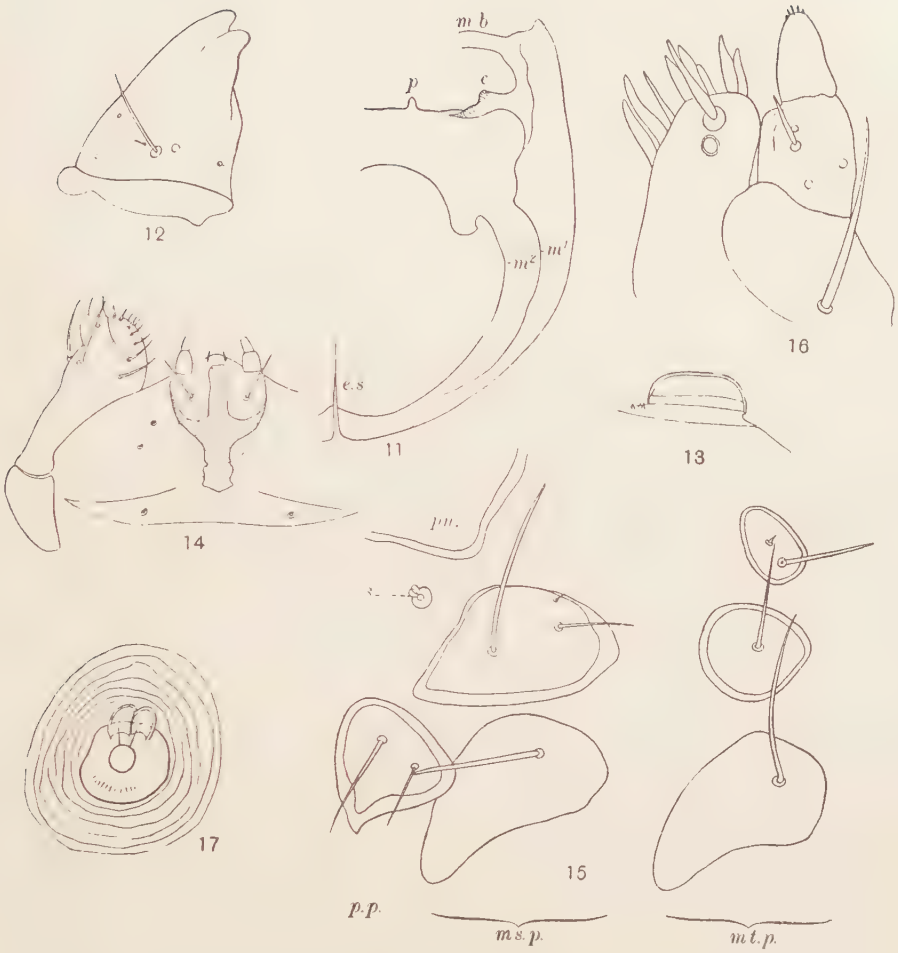
(*a*) *Host-plants of larva.*

Attempts to breed this species have not been successful, but larvae have been obtained from Dr K. W. Verhoeff, who has already given a brief summary of their characters in a synoptic table of five types of weevil larvae (17). He informs me that the larvae were always taken at the roots of *Populus nigra*; but this cannot be the sole host-plant, since Buckle (4) found the pupa beneath pasture and Mr R. Stenton informs me that he has reared the weevils from larvae found in the roots of dock (*Rumex*).

(*b*) *Mature larva.*

Length about 8·5 mm. Breadth at widest part (2nd abdominal segment) 2 mm., whence it tapers somewhat to the prothorax and posteriorly to the 9th abdominal segment.

Minute *spinulae* are present dorsally, ventrally and laterally on all segments of the body. But they are absent from the tubercles on which many of the setae stand and also from the chitinous plate of the



Phyllobius pyri L.

Fig. 11. Tentorium of larva (right half). ×65 *circa*. *c.*, condyle for articulation of maxillary cardo; *e.s.*, epicranial suture; *m.b.*, mandibular bar (broken); m^1., margin of epicranium; m^2., margin of tentorium; *p.*, anterior process.

Fig. 12. Mandible. ×65 *circa*.

Fig. 13. Antenna. ×212 *circa*.

Fig. 14. Labium and maxilla. ×65 *circa*.

Fig. 15. Pleural sclerites of thorax. ×65 *circa*. *ms.p.*, mesothoracic pleurae; *mt.p.*, metathoracic pleurae; *pn.*, pronotum; *p.p.*, prothoracic pleura; *s.*, thoracic spiracle.

Fig. 16. Maxillary mala and palp. ×212 *circa*.

Fig. 17. Thoracic spiracle. ×212 *circa*.

prothorax. In this species they are shorter and less coloured than in *P. urticae* and the character is therefore less obvious than in the latter species. They are longer and more spinous than the corresponding cuticular processes of *Otiorrhynchus sulcatus*.

The two teeth at the apex of the *mandible* are somewhat more curved and sharper than in *P. urticae*. The flange on the inner margin is also slightly more developed than in that species (Fig. 12). *Antenna* (Fig. 13) has the basal plate yellow, the principal sensory cone elliptical, transverse. Five or six short, somewhat erect, secondary cones are placed between the principal cone and the lateral margin. *Ocelli* absent. *Labrum* and epipharynx in general similar to those of *P. urticae*, but the taste-buds of the epipharynx are not tripartite, and appear rather to be bicuspid. A trident-like structure, supporting the terminal lobe of the *labium* and extending in a chitinous process to the submentum, is found here, as in *P. urticae*. The median process in this species, both on the terminal lobe itself and especially posteriorly, appears to be wider and to present generally a rather more truncate appearance (Fig. 14). The length of the segments of both *labial palps* and *maxillary palps* is about equal respectively. The *maxillary mala* (Fig. 16) is rounded on its inner margin like that of *P. urticae*, but the extremity, instead of being obtusely pointed, is almost straight and chisel-shaped, except that the lateral margins are rounded off. It has a similar deep excavation at the base adjoining the palpiger.

Chaetotaxy. The number and position of the setae of the body are very similar to those of *Phyllobius urticae*, as may be seen by reference to the table given below. The following points may, however, be noted: (1) on the prothoracic tergite no more than ten pairs of setae have been found; (2) the three epipleural setae of the mesothorax (Fig. 15), including one of very small size, are placed on an elongate fold or sclerite in an almost horizontal row behind the prothoracic spiracle instead of in an oblique row as in *P. urticae*; (3) the number of setae borne by the abdominal tergites (eight) commences to diminish with the 8th segment. Other

Somite	Tergite	Pleurite	Sternite
1st thoracic	10+10	2+2	7+7*
2nd „	6+ 6	4+4*	7+7*
3rd „	6+ 6	4+4*	7+7*
1st–7th abdominal	8+ 8	4+4	3+3
8th „	7+ 7	4+4	3+3
9th „	3+ 3	2+2*	2+2
10th „	—	—	2+2

* One seta on each side is extremely small.

small points of difference which may be noticed by comparison of the two tables appear to the writer of minor significance.

Spiracles (Fig. 17) situated in similar positions to those of *P. urticae*. As in that species the roof of the round atrium is formed of a thin membrane of chitin, in the centre of which is a small round opening. At the dorsal side of each spiracle, whether thoracic or abdominal, there are two sub-oval rudimentary chambers which project beyond the margin of the atrium and extend inwards towards the central opening. In the case of the thoracic spiracles these chambers have each an independent aperture in the form of a short slit in the roof of the atrium, extending to the central aperture, but no slit has been found in the abdominal spiracles. Minute trabeculae within the aperture are discernible in the thoracic spiracles under a high magnification. In one abnormal thoracic spiracle three of these chambers were found, though the corresponding spiracle on the other side had only two. The two closing levers are situated at some distance from the spiracle at the end of a comparatively long intermediate trachea. They are placed at right angles to one another.

(*c*) *Pupa*.

Described from a specimen received from Dr K. W. Verhoeff, Munich.

Length about 5 mm. Creamy white; the surface of the cuticle covered with pale microscopic spinulae. General form much as that of *P. urticae*. *Head* with five pairs of dorsal setae, arranged as in that species. *Pronotum* bearing an anterior oblique row of four setae placed on papillae, antero-laterally on either side. Behind these a pair of long conical papillae, one on either side of the median line, bearing a short, stiff, incurved, brown seta. In the middle of the pronotum another pair of short setae, one on either side of the median line. Near the posterior margin a transverse row of four setae placed on raised papillae, the median being larger than the lateral pair.

Mesonotum with a pair of short, brown, incurved setae on papillae, one on either side of the median scutum, also a fine and short seta, without any papilla, anteriorly, at the base of each elytron.

Metanotum with a stout brown seta, curved backwards, on either lobe of the metanotum, on a raised papilla.

Abdominal tergites 1 to 7 each with a transverse row of six short brown setae on raised papillae and a pair of short, fine, setae, also on short papillae, on either side laterally. 8th abdominal segment with a stout brown seta on a long, raised, papilla on either side of the medio-

dorsal line, another slightly shorter seta laterally and two very fine setae on ill-defined papillae in the pleura, all these setae being arranged in a transverse row. 9th abdominal segment has a pair of brown, incurved, spines at the extremity, each with a short, fine, seta at its base. The tergite bears a pair of short, fine, setae on scarcely raised papillae anteriorly. The oval-shaped anus is situated ventrally between the 9th tergite and sternite.

The ventral surface of the pupa is bare of setae, save for two somewhat long ones at the joint between the femur and tibia of each of the first pair of legs and a single one in the same position on the other legs.

3. **Polydrusus cervinus** L.

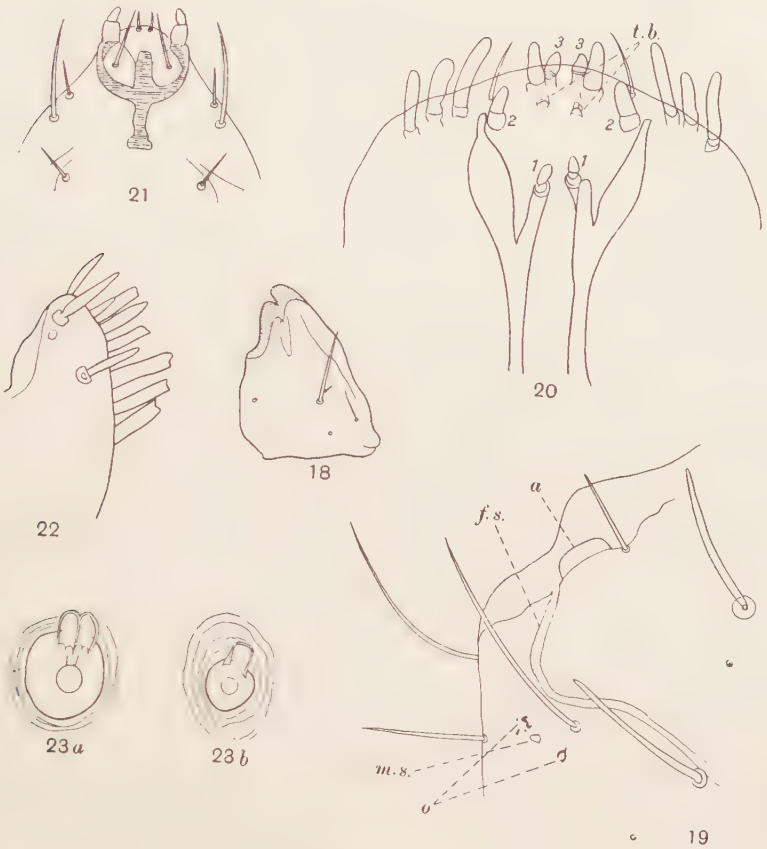
(*a*) *Biology.*

In the year 1847 Bouché (3) gave a brief description of a weevil larva found on the topmost branches of oak, eating the leaves and living in involucres of them. He attributed it to this species but, as was long ago suspected by Bargagli (2), evidently mistook some other for this, since it has now been found living in the soil.

At the end of May 1921 the present writer confined adults of this species in a pot, in which a young elm seedling had been planted. Neither ova nor young larvae were found, but in the following October two weevil larvae were discovered in the pot, similar to larvae taken in the open at the same time of year. Weeds had by this time grown on the surface of the pot and it is unsafe therefore to assume that the larvae had subsisted on the roots of the elm. In length they were then almost the same as at the time of pupation in March and April, *i.e.* 5 mm. In December larvae of about the same size have also been taken in the field, at the roots of grass in the neighbourhood of the beech trees, on the leaves of which the adults feed. There was no appearance of hibernation, though it is possible that the larvae may penetrate further into the soil in severe weather; but in any case the larvae probably take little food during the winter, since almost the whole increase in growth is made between the time of hatching and October. Many have been taken at the roots of Cocksfoot Grass (*Dactylis glomerata*), but others have been taken at the roots of other species of grasses and it is evident that grass roots of various species are their normal food.

From larvae taken in December 1922 and kept in a flower-pot containing sandy soil and a plant of *Dactylis* adults emerged successfully in April 1923. Adult beetles have also been reared from pupae taken in

the field in March and April. There can therefore be no doubt as to the identity of the species.



Polydrusus cervinus L.

Fig. 18. Mandible of larva. ×65 *circa.*

Fig. 19. Anterior angle of head, dorsal surface, to show position of ocelli (mandible removed). ×140 *circa. a.*, antenna; *f.s.*, frontal suture; *m.s.*, a micro-seta; *o.*, the ocelli.

Fig. 20. Labrum, with epipharynx as seen by transparence. ×212 *circa. 1, 2, 3,* epipharyngeal setae; *t.b.*, taste-buds.

Fig. 21. Labium, showing labial process. ×65 *circa.*

Fig. 22. Apex of maxillary mala. ×212 *circa.*

Fig. 23 *a.* Thoracic spiracle. ×212 *circa.*

Fig. 23 *b.* First abdominal spiracle. ×212 *circa.*

Pupation usually takes place at the end of March or beginning of April, but a few larvae may still be found up to the end of April. No precise data as to the exact duration of the pupal period are available,

but it appears to be of about 14–15 days, during which the pupa remains quiescent in a similar place to that occupied by the larva.

(*b*) *Mature larva.*

Length 5–6·5 mm., breadth 2 mm.

The dorsal surface of the body, apart from the setae, is almost smooth, but on the ventral surface minute, colourless, thorn-shaped spinulae are present from the prosternum to the 8th abdominal segment, visible only in prepared specimens and under high magnification. The arrangement of the micro-setae of the head differs little from that in *P. urticae*.

The *mandibles* (Fig. 18) are usually found in an eroded condition, showing scarcely any sign of apical teeth. When they are perfect, however, the teeth are developed somewhat like those of *P. urticae*, the outer one being produced a little further than in that species, while neither of the teeth is so much curved as in *P. pyri*. *Antenna* similar to that of *P. pyri* (Fig. 13); basal plate yellow; principal sensory cone elliptical. *Ocelli* two on each side of the head, each consisting of a small irregular mass of black granules (Fig. 19). They are situated near the anterior margin of each epicranial plate, one behind the other along the margin of the frontal suture. *Labrum* and epipharynx as seen from the under side are shown in Fig. 20. They are similar to those of *P. urticae*, except that the taste-buds in this species are irregularly polygonal and angular, while in *P. urticae* they are tripartite and rounded.

The four setae at the base of the *clypeus* are in proportion a little longer than those of *P. urticae* and *P. pyri*.

The *submentum* is not strongly developed but appears to be more rounded than in *P. urticae*. The terminal lobe of the labium is almost round. It bears the usual trident-shaped thickening of the chitin, consisting of a stripe in the median line and one on either side, arising at the base of the palp and coalescing with the median stripe before continuing in a chitinous process to the margin of the submentum. In this species the process is broadened at the base, so that it forms an inverted T (Fig. 21). The two segments of each *labial palp* are nearly equal in length, as also are those of the *maxillary palps*.

The *mala* of the maxilla is bluntly pointed at the apex and slightly flattened for some distance between the apex and the inner margin (Fig. 22). The ventral surface of the mala bears two stiff setae in place of the one found in *P. urticae* and *P. pyri*. Inner margin at the base, adjoining the palpiger, scarcely excavate.

Chaetotaxy, Very similar to that of *P. pyri*. The three epipleural setae of the mesothorax, however, are placed in an almost oblique line behind the prothoracic spiracle, as in *P. urticae*.

Somite	Tergite	Pleurite	Sternite
1st thoracic	10+10	2+2	7+7
2nd ,,	6+ 6	4+4	7+7
3rd ,,	6+ 6	4+4	7+7
1st–6th abdominal	8+ 8	4+4	3+3
7th ,,	7+ 7	4+4	3+3
8th ,,	5+ 5	4+4	3+3
9th ,,	4+ 4	1+1	2+2
10th ,,	—	—	2+2

Spiracles of thorax (Fig. 23 *a*) with two chambers, those of the abdomen (Fig. 23 *b*) with only one, each of which is directed towards the dorsal surface. Those of the thorax resemble the spiracles of *P. pyri*, the chambers being of the same simple form and having a slit at the surface of each extending to the circular aperture in the roof of the atrium, but they differ in that each chamber of *P. cervinus* has a minute tooth projecting inwards at its mouth. Each abdominal spiracle possesses only one chamber, which is without any slit at the surface or any tooth. No trabeculae are visible from the exterior. The intermediate trachea between the spiracle and the closing apparatus is comparatively long.

(*c*) *Pupa*.

Length 4·5 mm. Creamy white, covered with minute spinulae. General appearance (Fig. 24) as in foregoing species.

Head with nine pairs of setae on the dorsal surface—three above the

Fig. 24. *Polydrusus cervinus* L. Pupa. ×12.

imaginal eyes, three between them and three on the sides of the rostrum, forming a longitudinal row of three setae on either side of it.

Pronotum with sixteen setae on raised papillae, arranged as in the pupa of *P. pyri*.

Mesonotum with a transverse row of four setae anteriorly, followed

on either side by two setae, which, with the anterior median seta, form an oblique row of three setae on either side of the median line.

Metanotum with two short anterior setae, one on either side of the mediodorsal line, followed by two setae placed on an oval tubercle on either side.

The *leg-sheaths* bear two setae each, one a little longer than the other, at the joint between femur and tibia.

Abdominal tergites 1–7 have each a row of six short, brown, recurved spines in a transverse row. Pleurae with two, a coarser and a finer seta on each side, anterior to the tergal setae of the segment.

8th tergite with only two spines, one of the two pleural setae being strongly developed in this and the preceding segment.

9th segment with the usual two stout brown incurved spines (pseudocerci) at the apex dorsally and with three fine setae, forming an oblique row on each side ventrally.

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(*Received November 18th*, 1925.)

OBSERVATIONS ON THE BIOLOGY OF *TYLENCHUS DIPSACI* (KUHN) BASTIAN, AND ON THE OCCURRENCE OF BIOLOGIC STRAINS OF THE NEMATODE

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Introduction.

The stem eelworm, *Tylenchus dipsaci* (Kuhn) Bastian, causes severe injury to a variety of both wild and cultivated plants, on the latter of which it is a pest of considerable economic importance. In the following paper the writer has made an attempt to add to our knowledge of the normal biology of this nematode. The small size of the eelworms and the difficulty experienced in keeping them alive under artificial conditions make the task of accurate observation a difficult one: nevertheless, it has been possible to arrive at certain definite conclusions, which are stated in the paper. They are followed by a discussion of the existence of Biologic Strains in this species. A morphological description of the eelworm has not been included, as this has been made by many previous workers, notably by Ritzema Bos (8) and Marcinowski (7).

Biology.

Life-history. The life-history is essentially the same in all those host plants, which the present writer has had an opportunity of studying. The eelworms breed with great rapidity under favourable conditions. Temperature seems to affect them only indirectly in so far as it controls plant growth. Breeding is usual only in succulent and rapidly growing, or in storage tissues. For example, all stages of the worm from egg to adult may be found in a state of activity in narcissus bulbs throughout the winter months. On the other hand, oat seedlings infected in late autumn were not found to contain any eggs, or to show any appreciable increase in the number of eelworms present, until the latter end of March, plant growth having by then become more rapid.

As the worms increase in numbers, hypertrophy, distortion, splitting and finally death of the invaded plant tissues take place, the eelworms passing into fresh portions of the plants as the decay spreads. At this advanced stage of attack, numbers of the parasite leave the host plant and enter the ground in search of a further supply of food. It frequently happens that this retreat is cut off by quantities of decaying or dried tissue. In this case if the infested portions dry up, the worms become quiescent, in which state they are able to persist for a very considerable time (Goodey (5)). With reference to this, it was noted that there does not appear to be any attempt to avoid desiccation, this being so even when fresh portions of the plant, in which other worms are still breeding, are adjacent. Migration to these areas would apparently have been an easy procedure.

Numbers of the worms are sometimes trapped in the decaying tissue which, as a result of external conditions remains moist for an extended period after death. This is of particularly common occurrence in narcissus bulbs situated below soil level. The death of eelworms so trapped takes place usually in a very short time, moist decaying tissue being apparently highly toxic to this particular nematode. At the same time, non-parasitic genera of nematodes, which are almost invariably present under such conditions, continue to flourish. Experimental confirmation of this apparent toxicity was obtained by placing active adults in a solution of decayed bulb in distilled water. Death usually took place within a few hours. That death in these cases was not due to drowning is certain, as the writer has found no great difficulty in keeping adults alive in distilled water for as long as five weeks, death ultimately appearing to be due to starvation.

The nematodes at the time of entering the soil, or alternatively of becoming quiescent are exceedingly opaque, being packed with stored food matter; on which reserve they undoubtedly rely until they find a fresh host. There is no apparent change in the desiccated forms until they are revived by the application of moisture. The revival is most easily effected by complete immersion in water and occurs after a period varying in length according to the time during which they have remained inactive. These revived nematodes, if kept in distilled water, will remain active and will gradually utilise their reserve of food until they become almost transparent. A similar phenomenon has been observed previously in the case of the larvae of *Tylenchus tritici* by Byars (1).

While the foregoing holds good to a limited extent for all stages of *T. dipsaci*, it is the last larval stage, in which some sexual differentiation

is to be observed, that appears to persist longest under these conditions, as shown by Goodey in the case of *T. dipsaci* from narcissus (5). Small larvae and adults do not seem to be adapted to carrying such a large reserve of food and are therefore unable to remain active for such an extended period in the absence of a suitable host.

Symptoms of attack. Attacked plants may usually be detected by the characteristically distorted and deformed appearance which they assume. The plant as a whole is generally dwarfed, this dwarfing being accompanied by considerable hypertrophy of certain portions of the plant. On teasing out portions of the damaged tissue in water, enormous numbers of the nematodes in all stages of development may be found.

The roots are never entered. The basal portions of the stem and the soft tissues in the vicinity of the growing points are the positions primarily attacked. Later the petioles and leaves are in turn invaded and become distorted, swollen and blistered. If the plant is small when attacked it usually succumbs, unless growing in a particularly favourable situation. Cereal hosts are, however, frequently able to grow away and to throw off the attack as the tissues become less succulent and so too tough for the eelworms to penetrate. Nevertheless, such plants will sustain the loss of most of their early tillers and will not become so vigorous as those which have escaped infection. Chlorosis, splitting and blistering of the leaf and stem are frequent symptoms in cases of heavy infestation of a plant previously vigorous.

Bulbs, onions, etc., frequently constitute the host plant and in these cases numbers of the worms are to be found in the bulb tissues as well as in the leaves and stems. Chlorosis is again a frequent symptom, as is the distortion and splitting of the foliage; the latter characters, however, are less marked on this type of host. Potatoes are also sometimes attacked. In these cases the eelworms occur in the tubers only and not in the foliage, which presents a normal appearance (Goodey (4)).

Sources of infection. Consideration of the above observations indicates two main sources of infection of potential host plants.

The first consists of those eelworms which remain in a desiccated state in the dead host plant. In this condition they can persist for a period amounting to several years (Goodey (5)). Until the advent of moisture no sign of life is visible and no call is made on the stored food matter.

The second source comprises those eelworms which have escaped directly into the soil. These are not able to become quiescent as long as they are in a moist environment, and while they remain in the soil this is likely to be the case. They are unable to feed until they have

found a fresh host. The length of time during which this enforced starvation can be withstood has not yet been determined, but from experiments now in progress it appears to be of shorter duration than has generally been thought to be the case.

Yet a third mode of infection, in reality an extension of the first, has been found to occur occasionally on the oat. Examination of a large number of samples of seed from badly infected fields has revealed the occasional occurrence of quiescent *T. dipsaci* larvae beneath the pales of the seed. Seedlings grown from these sources have produced a small percentage of infected plants. While the number of cases found is so small as to suggest that the occurrence is accidental, careful watch should be kept for any development in this direction, as it has been found recently in America (Godfrey (3)) that seed dissemination is the normal mode of spread adopted by this eelworm when occurring on certain Compositae.

Method of entering host. Observation has shown that the immediately pre-adult larval form is the most resistant to unfavourable conditions. It is in all probability this form which is chiefly responsible for the spread to new hosts under field conditions.

The writer has frequently attempted to inoculate seedlings with adult nematodes, by means of a glass pipette, but with little success. The nematodes were placed on the plants in a water drop and in almost every case made no attempt to enter the plant, but became quiescent as the water evaporated. Larvae have on numerous occasions been induced to enter plants with comparative ease under identical conditions.

It seems certain that entry is not made through the roots, but probably takes place in the vicinity of the leaf bases and the growing points where the tissues are softest. While it has not been possible so far to observe a nematode in the process of entering a plant, examination of seedlings, placed in heavily infested sand for 24 hours, has revealed numbers of the worms congregated, ectoparasitically, between the leaf bases and in the vicinity of the growing point. Plants examined after 48 hours' exposure to infection have been found to contain the nematodes, actually in the tissues in these areas, and it is hoped that it may be possible in the near future to elucidate the exact mode of entry employed.

The so-called piercing style in the buccal cavity may be used for this purpose, but it seems exceedingly doubtful if this is ever exserted (Goodey (7)). The writer has certainly never seen it so used. There seems, however, to be no reason why the worm could not exert sufficient mechanical pressure to gain an entry in the softer parts of the plant.

Biologic Strain Theory.

Of recent years attention has been drawn to the fact that there appear to exist numerous biologic strains of this and other plant parasitic nematodes, these strains being identical with one another from the morphological view-point but each particularly adapted to parasitising some individual host plant, and unable or at least unlikely to attack available hosts dissimilar from those on which they have previously been feeding. Much of the existing information on this subject has recently been collected and discussed by Steiner (9).

Considerable economic significance attaches to a definite proof of the existence of such strains, as it would then be possible to determine the degree of safety with which possible alternative hosts might follow a crop previously attacked by the eelworm.

Field observations. While little experimental data exist regarding the occurrence of such biologic strains, field observations certainly indicate the existence of at least a very marked preference for some particular hosts.

To quote but a few examples. *T. dipsaci* is a serious pest of the strawberry in some parts of America (Byars (2)). In certain areas in this country, strawberry and narcissus bulbs are the two main smallholding crops, and have been alternated on the same ground for many years. No case of attack on the strawberry has ever been seen by the writer in the course of extensive observations, while the occurrence of a heavy infection of the bulbs is common.

In another bulb area, oat, a well-known host of this eelworm, is frequently grown in the vicinity of infested bulb fields, and even on ground which has probably ceased to grow bulbs owing to the depredations of the pest. No case of attack on the oat has so far been found and enquiries indicate that it is unknown as a host in this area.

A third instance may be quoted. Oat fields severely attacked are frequently found to contain clover, another favourite host, in a flourishing condition. To mention a case in point, a field containing Abundance oat, so badly attacked as to fail completely, the yield being about 8 bushels per acre, as against a normal yield of 45 bushels on the field in question, produced in the autumn a good crop of red clover. No sign of attack was to be found on this, either at the time or subsequently.

Field experiments. Field experiments tend to confirm these observations. In 1924 strips of 12 varieties of red clover were sown across a field which, for the past two seasons, had carried oat crops heavily

attacked by the eelworm. Stray oat seedlings coming up during the third season were heavily infected, but periodic examination of the clover failed to reveal any signs of attack.

The experiment was duplicated on a very similar field in another locality. This field had also carried infested oats for two seasons. In this case the clovers used were, Single Cut Cowgrass, English Red, Cornish Marl, Alsike, Wild White, Dutch White and Giant Hybrid. Periodic examination again gave negative results.

In the third experiment, large numbers of heavily infested bulbs were broken up and broadcasted on a clover ley. This ley had been used as a variety trial and as a result contained a very large number of different clovers. No sign of attack was to be observed within six months, during which period several hundred plants were examined. A repetition of this experiment is perhaps desirable, as the bulk of the bulbs used had been stored in the laboratory for a considerable period, and it is possible that the vitality of the eelworms may have been impaired, as only about 25 per cent. of them were found to revive after immersion in water. Nevertheless, re-infection of narcissus bulbs was successfully carried out with identical material.

Cylinder experiments. In order to obtain more exact data, an attempt was made to transfer strains of the nematode from one host to another under controlled conditions.

The experiments were commenced in earthenware flowerpots; these, however, proved unsatisfactory for several reasons and were abandoned in favour of zinc cylinders. The cylinders were 2 ft. deep, 9 in. in diameter and open at each end. They were sunk in the ground to within about 2 in. of their tops and filled with soil, which was not treated but assumed to be free from *T. dipsaci*. As the control cylinders were without exception free from attack, this assumption was presumably justified.

Each experiment was repeated in a number of cylinders, and was further duplicated by repeating with similar materials from other sources. With the exception of one or two negative results no discrepancies occurred.

Some difficulty was experienced in obtaining a number of races of *T. dipsaci* whose histories were known over a series of years; however, four such races were procured, as also were three races whose previous histories were unknown.

The procedure in each case was to infect heavily a number of cylinders with a known race of the eelworm, the number of cylinders not being constant, but varying with the quantity of material available. Various

alternative hosts were then sown, including in most cases some of the original host of the particular race present. The plants were examined at monthly intervals, commencing one month after sowing, and if three consecutive examinations gave negative results the plants were considered to be immune and the experiment discontinued. When narcissus bulbs were used as alternative hosts, early examinations proved useless, and a period of several months was allowed to elapse before the bulbs were examined. One alternative host only was sown in each cylinder, as it was not considered desirable to complicate the results obtained by the introduction of a choice of food plants. Uninfected control cylinders were in all cases sown and periodically examined.

Description of experiments. A. A strain which had bred continually in narcissus bulbs, for a period of at least three years.

The supply of infected material was limited and it was only possible to infect six cylinders. Oat and clover were sown over infected material and the seedlings examined at monthly intervals for signs of attack, the first examination being made one month after sowing. Neither was attacked and the experiment was discontinued after three months.

B. A narcissus strain, which had bred continually in the one host for at least four years.

A series of fifteen cylinders were infected with the strain in March. Narcissus bulbs planted in a number of the cylinders were found to become heavily infected. Oat and clover were sown in the remaining cylinders and on examination one month after sowing, a limited number of the plants were found to contain a few eelworms. No eelworms remained in the oats at the time of the second examination but a few persisted in the clover. At the end of three months no trace of attack could be found on either.

C. A strain living on imported narcissus bulbs. The previous history of this strain was unknown.

Fifteen cylinders were used and the experiment was a duplicate of that carried out with strain B. Narcissus was again found to become infected, about 75 per cent. of the bulbs used containing eelworms in all stages of development three months after exposure to infection. The eelworms were fairly numerous in the oat at the time of the first examination, less so at the second and entirely absent at the third. No deleterious effect on the plants could be detected. In the case of the clover, small numbers of the worms were found at each examination. No breeding was observed, but the persistence in this host for a number of months is worthy of note.

D. A strain attacking onion, the previous history again unknown.

The supply of infected material being limited, it was only found possible to use nine cylinders. Onion was sown in three of these and in two out of the three typical attacks developed. In the third very few plants suffered. Oat and clover were sown in the remainder and no attack took place on either. It is proposed to attempt infection of narcissus bulbs when further supplies of an onion strain become available.

E. A strain occurring in potato tubers, previous history unknown.

The supply of infected material was again very limited, and it was found possible to use only seven cylinders. Infection of healthy tubers was easily effected. Oat was the only other potential host used and although the nematode was present in numbers in the seedlings at the times of the first and second examinations, there was no sign of attack or trace of the worms at the end of three months.

F. A strain occurring in oat. The field from which this strain was taken had contained, the year previously, cocksfoot heavily infected, and the inference, borne out by experimental results, is that the one strain was equally at home on both hosts.

Fifteen cylinders were used and attempts made to infect oat, cocksfoot, clover and narcissus. Narcissus was not attacked, a few eelworms were, however, found near the edges of the outer bulb scales. Clover was entered, but by the third examination no worms or signs of attack were to be found. Oat and cocksfoot were heavily attacked and by the end of three months many of the plants were dead, as a result of the severity of the infestation.

G. A strain which had occurred on oat for two consecutive years.

Sixteen cylinders were used and oat, clover and narcissus again employed. Narcissus bulbs were not attacked. Early examinations again showed eelworms to be present in the clover, but no attack developed subsequently. The oats were heavily attacked almost at once and many of the plants killed.

Discussion of experiments. Consideration of the foregoing data and inspection of the experimental results seem to provide further evidence in favour of the acceptance of the biologic strain theory.

Re-infection of the original host was found to occur almost without exception, and at the same time there is very definite evidence that difficulty is experienced by the nematode in attacking a host other than the one on which the strain has previously been feeding.

Entry into the plant was frequently effected, the difficulty seeming to consist rather of an inability to become established once such entry

had been made. It was further noticed that the eelworms taken from these plants were highly transparent, resembling ones which had been starved in distilled water for extended periods, the alimentary canal being empty in both cases. The worms were furthermore exceedingly sluggish and their whole condition indicated that they had not fed since entering the host. In some cases they were found to disappear from the plants after a few weeks, having probably died, but in others they persisted for periods extending to months and in such cases might reasonably be expected to adapt themselves eventually to the new host.

The experiments described in this paper certainly indicate a very marked rigidity as regards the disability to cause injury to another host. However, further experiments now in progress show that such rigidity is not quite invariable, as is borne out by the fact that one of the strains used in the first series, namely G, was equally at home on both oat and cocksfoot, although apparently unable to attack clover. Furthermore, the ability to transfer from one host to another probably depends very largely upon the length of time that one particular host has been utilised, the experiments now in progress giving some indication that this is the case. The more generalised forms do not, as a rule, appear to cause such severe injury to plants attacked as do those which have concentrated on the one host.

The practical import of such information as can be deduced from the foregoing is perhaps, at this stage, not very conclusive; however it appears that, while, in the case of attack by one strain on a particular host it would be safe to follow with another crop which is a potential host of the eelworm, it might so happen that the strain present was a generalised form, in which case the following crop would suffer accordingly. The principal factor influencing the behaviour in this direction is, as already mentioned, almost certainly the length of time during which the previous host has carried the strain and in cases where such information is available the course of action may be determined accordingly. In all probability, if it is found that the previous host has been attacked by the strain for a period of two years or more, the attack on a potential host following the infected crop would be so slight as to be negligible.

Summary.

1. The stem eelworm, *Tylenchus dipsaci* (Kuhn) Bastian, is a severe pest on a variety of crops.

2. Usual symptoms of attack constitute dwarfing, distortion and frequently chlorosis of the host plant.

3. The biology of the eelworm is essentially the same in nearly all host plants.

4. Infection may take place immediately after leaving the previous host, or after a period of enforced quiescence.

5. Examination of available data indicates that, while biologic strains or races of the eelworm do exist, these strains show varying powers of adaptation to other host plants.

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(*Received January 18th*, 1926.)

ON THE OCCURRENCE OF *CENTOR MYOPINUS* MEIG. AS A PEST ON OAT

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Certain flies, members of the family Chloropidae, constitute well-known pests of cereals. The damage caused is attributable to the habits of the larvae, which tunnel the young shoots of attacked plants. Notable examples of such pests are *Oscinella frit* Linn. which attacks principally the oat, and *Chlorops taeniopus* Meig., the Gout Fly, which damages barley and other cereals.

The fly under consideration, namely *Centor myopinus* Meig., bears some superficial resemblance to *Chlorops taeniopus* but may be at once distinguished from it by the absence of yellow stripes on the dorsal surface of the thorax. It may be briefly described as follows: Head hemispherical. Eyes red. Arista pubescent. Upper part of back of head, a large vertical triangle, dorsal region of the thorax and the whole of the abdomen, dark metallic brown and shining. Frons, face, under side of head, most of the under side of thorax and the legs excepting the anterior tarsi, yellow. Anterior tarsi fuscous to black. A short but strong and slightly curved apical spine beneath the middle tibiae. Legs and wings rather short. Length of adult 3 to 4 mm.

The fly first came under observation in May 1925, when a field of spring sown oats was observed to be dying off in considerable patches. Superficially the damage much resembled that usually occasioned by the larvae of *Agriotes* spp., or wireworm. Examination of the plants, which were approximately 3 in. in height at the time, revealed the presence of numerous Dipterous larvae. These were tunnelling the centre of the tillers and by consuming the growing points were rapidly destroying them. Attacked plants could be at once detected from some distance away by their typical yellow appearance. In most cases several larvae were present in each plant. The oat being a poor tillering variety was in consequence unable to grow away from the attack as a free tillering one might have done. The larvae were found to pupate in the base of the injured shoot at about ground level. Adult flies commenced to

emerge in the middle of June, emergence continuing until the end of the month. The sexes occurred in approximately equal numbers, males living for about 10 days in confinement, females somewhat longer.

The adult flies were confined in large glass cylinders, covered in muslin. These cylinders were placed over pots containing oat plants in early stages of development. Sugar and water were supplied and the flies were observed to feed at intervals. Copulation took place freely, but there was no sign of oviposition. The females frequently rested on the basal regions of the plants but no ova were to be found and subsequent examination of the plants failed to disclose any larvae.

Certain of the Chloropidae, whose detailed life-histories have been studied, have been found to utilise different host plants or different portions of the same host, for their winter and summer broods respectively. It would appear that failure to obtain oviposition was in all probability due to the lack of the provision of a suitable host plant. The oat would not be found in early stages of development under field conditions and it is quite possible that this insect may utilise some other host for its summer broods. In support of this hypothesis it may be mentioned that a few of the flies were obtained by sweeping in the original field during June, but no further attack on the plants growing there was to be observed.

In view of the serious nature of the injury occasioned in the one case recorded, as many as 50 per cent. of the plants in affected areas of the field being killed outright, and many others being permanently checked, it is proposed to investigate the life-history of the fly in detail when further material becomes available.

(*Received October 26th*, 1925.)

STUDIES ON CARBON DIOXIDE PRODUCTION IN SOIL AND SOLUTION[1]

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(With 2 Text-figures.)

I. Production of Carbon Dioxide by *B. prodigiosus* in Mineral Nutrient Solution with varying Amounts of Different Sugars.

Introduction.

The rate of evolution of carbon dioxide from a soil depends upon the power of the latter to decompose the organic matter, and soils in general show a great variation in this respect. The evolution of carbon dioxide from a soil is therefore very commonly used as an index of soil fertility.

The rate of carbon dioxide production is governed by many factors, and the nature of the micro-organic population of the soil is considered to be one of them. It was for this reason thought of interest to investigate the action of some of the saprophytic organisms on various sugars, and *B. prodigiosus* was chosen as a suitable organism.

Experimental.

After various mineral nutrient solutions had been tried it was found that the one given below was most suitable for the growth of *B. prodigiosus*.

Na_2SO_4	2·5	$CaCl_2$	0·01
NaCl	2·5	$(NH_4)_2SO_4$	2·00
K_2HPO_4	2·5	Water	1000

The procedure adopted in all the experiments was as follows: The solutions in the various experiments were sterilised by intermittent sterilisation for three days, after which they were inoculated with pure culture of *B. prodigiosus*, using the same number of organisms for each set of experiments. The flasks were then connected to Pettenkoffer's

[1] This work was carried out some years ago under the direction of Mr C. M. Hutchinson, B.A., C.I.E., Imperial Agricultural Bacteriologist, Pusa (India), and the writer takes this opportunity to express his sincere gratitude to him for his kind interest and valuable advice throughout the course of this work.

tubes containing known amounts of standard barium hydroxide solution and two litres of air, freed from carbon dioxide by passing it over caustic soda, was aspirated through them every day. The amount of carbon dioxide liberated was determined by titrating the barium hydroxide with standard acid, using phenolphthalein as indicator. Carbon dioxide free distilled water was used for titration and the figures for carbon dioxide were calculated after allowing for the necessary blanks.

Exp. 1. Effect of different volumes of liquid containing the same amount of mineral nutrients and glucose on the amount of carbon dioxide produced.

The following amounts of nutrient solution and glucose were employed:

(*a*) 100 c.c. nutrient solution + 0·2 gm. glucose.

(*b*) 100 c.c. nutrient solution diluted to 400 c.c. by the addition of distilled water + 0·2 gm. glucose.

The results obtained are given below (Table I).

Table I.

Showing the amount of carbon dioxide in milligrammes evolved on successive days.

	(*a*)	(*b*)
	2·86	Nil
	21·23	Nil
	25·96	41·36
	16·06	35·09
	11·33	17·16
	4·73	12·43
	3·63	8·03
	3·30	4·40
	2·20	3·30
	2·75	1·65
	2·75	1·65
	3·30	2·75
	3·30	2·75
Total	103·40	130·57
Amount of glucose found in the solution after the experiment	23·2 mg.	24·4 mg.
Amount of carbon dioxide equivalent to the quantity of glucose found	34·1 „	35·86 „
Amount of carbon dioxide equivalent to the quantity of glucose added	294·0 „	294·0 „
Amount of carbon dioxide unaccounted for	156·50 „	127·57 „
Amount of *N*/10 alkali required to neutralise the acidity in the solution after the experiment	7·0 c.c.	9·64 c.c.

The results show that high dilution helps the organisms to utilise the sugar weight for weight.

Exp. 2. Carbon dioxide production in equal volumes of mineral nutrient solution with different amounts of sucrose. The following solutions were employed in this experiment:

(*a*) 100 c.c. nutrient solution + 0·1 gm. sucrose.
(*b*) 100 c.c. ,, ,, + 0·2 gm. ,,
(*c*) 100 c.c. ,, ,, + 0·3 gm. ,,
(*d*) 100 c.c. ,, ,, + 0·4 gm. ,,

The results obtained are given below (Table II).

Table II.

Showing the amount of carbon dioxide in milligrammes evolved on successive days from varying quantities of sucrose.

	(*a*)	(*b*)	(*c*)	(*d*)
	18·76	7·70	17·60	16·50
	25·30	36·30	61·60	88·00
	12·10	20·90	28·60	44·00
	6·16	8·36	13·86	25·52
	3·30	5·50	9·68	16·50
	0·60	0·66	6·16	6·16
	1·10	—	—	—
	1·10	1·76	5·06	6·16
	—	1·32	3·96	3·30
Total	68·42	82·50	146·52	206·14
Total invert sugar present after the experiment	12·0 mg.	65·0 mg.	100·0 mg.	135·0 mg.
Carbon dioxide equivalent to the amount of invert sugar present	17·55 ,,	95·48 ,,	146·30 ,,	197·12 ,,
Carbon dioxide equivalent to the quantity of sugar added	154·00 ,,	308·00 ,,	462·00 ,,	616·00 ,,
Carbon dioxide unaccounted for	68·03 ,,	130·02 ,,	169·18 ,,	212·74 ,,
Amount of *N*/10 alkali required to neutralise the acidity in the solution after the experiment	6·0 c.c.	7·0 c.c.	8·0 c.c.	10·0 c.c.

Exp. 3. Carbon dioxide production in equal volumes of mineral nutrient solution with different amounts of laevulose.

The plan of this experiment was the same as in Exp. 2 except that laevulose was used instead of sucrose. The results are given below (Table III).

Table III.

Showing the amounts of carbon dioxide in milligrammes evolved on successive days from varying quantities of laevulose.

	(*a*)	(*b*)	(*c*)	(*d*)
	4·40	6·60	7·70	6·60
	31·68	50·60	55·00	46·40
	16·50	26·40	33·00	70·40
	9·46	13·86	15·62	25·08
	8·36	7·26	12·76	18·92
	6·16	3·96	8·36	11·66
	5·06	4·40	6·16	10·56
	3·96	1·76	5·06	7·26
Total	85·58	114·84	143·66	196·88
Laevulose present in the solution after the experiment	10·0 mg.	55·0 mg.	80·0 mg.	130·0 mg.
Carbon dioxide equivalent to the amount of laevulose present	14·7 ,,	80·85 ,,	117·60 ,,	181·10 ,,
Carbon dioxide equivalent to the quantity of laevulose added	147·0 ,,	294·0 ,,	441·0 ,,	588·0 ,,
Carbon dioxide unaccounted for	46·72 ,,	98·31 ,,	179·74 ,,	210·02 ,,
Amount of $N/10$ alkali required to neutralise the acidity in the solution after the experiment	6·0 c.c.	7·0 c.c.	9·0 c.c.	9·0 c.c.

Table IV.

Showing the amount of carbon dioxide in milligrammes evolved on successive days from varying quantities of maltose.

	(*a*)	(*b*)	(*c*)	(*d*)
	1·54	3·30	2·86	3·52
	0·57	1·32	3·08	2·42
	0·57	1·32	1·32	3·52
	0·57	1·32	1·32	3·08*
	0·57	1·32	1·32	3·08
	0·57	1·32	1·32†	3·41
	—	—	20·79	—
	0·77	0·99	28·82	1·65
	—	—	14·82	—
	—	—	8·69	—
Total	5·16	10·89	—	20·68
Maltose present in the solution after the experiment	78·20 mg.	166·0 mg.	—	352·1 mg.
Carbon dioxide equivalent to the quantity of maltose found	114·60 ,,	244·0 ,,	—	517·44 ,,
Carbon dioxide equivalent to the sugar added	147·0 ,,	294·00 ,,	—	588·00 ,,
Carbon dioxide unaccounted for	27·2 ,,	39·0 ,,	—	49·0 ,,
$N/10$ alkali required to neutralise the acidity in the solution after the experiment	6·0 c.c.	6·0 c.c.	—	9·0 c.c.

* One loopful of fresh *B. prodigiosus* culture added.

† Four c.c. of 5 % sterilised glucose solution added.

Exp. 4. Carbon dioxide production in equal volumes of mineral nutrient solution with different amounts of maltose. The solutions employed in this experiment were the same as in the previous experiments except that maltose was used in place of laevulose. The results obtained are given above (Table IV).

Exp. 5. Carbon dioxide production in equal volumes of mineral nutrient solution with different amounts of lactose. The solutions employed in this experiment were the same as in the previous one except that lactose was used instead of maltose. The results obtained are given below (Table V).

Table V.

Showing the amount of carbon dioxide in milligrammes evolved on successive days from varying quantities of lactose.

	(*a*)	(*b*)	(*c*)	(*d*)
	1·10	1·54	1·98	5·06
	2·05	1·89	1·98	2·42
	2·05	1·89	1·70	5·72
	2·05	1·89	1·70	2·53*
	1·59	1·89	1·70	2·53
	1·59	1·89	1·70†	2·97
	—	—	17·49	—
	—	—	29·48	3·19
	3·19	3·19	14·08	—
	—	—	11·99	—
Total	13·44	14·18	—	24·42
Lactose present in the solution after the experiment	90·0 mg.	175·8 mg.	—	303·0 mg.
Carbon dioxide equivalent to the lactose found	132·30 „	258·7 „	—	445·4 „
Carbon dioxide equivalent to the sugar added	147·0 „	294·0 „	—	588·0 „
Carbon dioxide unaccounted for	1·3 „	21·0 „	—	118·0 „
N/10 alkali required to neutralise the acidity in the solution after the experiment	9·0 c.c.	7·0 c.c.		7·0 c.c.

* One loopful of fresh *B. prodigiosus* culture added.

† Four c.c. of sterilised glucose solution added.

Exp. 6. Carbon dioxide production in equal volumes of mineral nutrient solution with different quantities of glucose. The general plan of this experiment was the same as in the previous ones, glucose being employed as a source of organic food. In addition to the usual estimation of carbon dioxide the numbers of organisms were estimated by making daily counts by the plate method. One hundred organisms were initially introduced in all the flasks as an inoculum. The results obtained are given below (Table VI).

Table VI.

Showing the amount of carbon dioxide in milligrammes evolved on successive days from varying quantities of glucose and the numbers of organisms per c.c. per day.

Dates	100 c.c. nutrient solution only		100 c.c. nutrient solution +0·1 gm. glucose		100 c.c. nutrient solution +0·2 gm. glucose		100 c.c. nutrient solution +0·3 gm. glucose		100 c.c. nutrient solution +0·4 gm. glucose	
	Carbon dioxide	Number of organisms per c.c. of the solution in millions	Carbon dioxide	Number of organisms per c.c. of the solution in millions	Carbon dioxide	Number of organisms per c.c. of the solution in millions	Carbon dioxide	Number of organisms per c.c. of the solution in millions	Carbon dioxide	Number of organisms per c.c. of the solution in millions
8. ix. 15	1·1	0·000384	Nil	0·001600	Nil	0·110	Nil	0·014575	Nil	0·002400
9. ix. 15	1·1	0·847	3·96	84·5	42·46	418·0	24·09	345·0	13·09	270·0
10. ix. 15	1·21	1·92	33·66	528·0	32·90	860·16	45·76	921·6	59·30	782·08
11. ix. 15	1·43	1·60	21·89	748·0	14·96	554·4	21·89	748·8	27·39	783·36
12. ix. 15	1·43	0·80	8·36	514·0	4·73	827·2	16·06	959·2	19·36	947·52
13. ix. 15	1·87	0·720	3·63	748·0	2·53	712·8	6·93	1584·0	8·03	1672·0
14. ix. 15	0·82	0·409	2·53	563·2	3·63	797·6	4·73	1031·0	5·83	1170·2
15. ix. 15	0·80	0·354	2·20	514·08	3·30	624·96	3·30	825·6	3·30	1056·0
16. ix. 15	0·80	0·341	2·20	399·36	3·30	668·16	3·30	685·4	3·30	921·6
17. ix. 15	0·88	0·40	2·20	500·8	1·65	691·2	3·30	880·0	8·80	1031·68
18. ix. 15	0·88	0·416	2·20	483·84	1·65	633·6	3·30	704·0	8·80	1164·8
19. ix. 15	1·1	—	2·75	—	2·20	—	3·52	—	16·50	1105·7
20. ix. 15	1·1	0·642	2·75	262·08	2·20	362·88	3·52	528·0	12·10	1113·8
21. ix. 15	—	—	—	—	—	—	—	—	7·70	1000·0
Total	14·52	—	88·33	—	115·51	—	139·7	—	193·50	—
Amount of glucose found in the solution	Nil		Nil		40·0 mg.		80·0 mg.		Nil	
Amount of carbon dioxide equivalent to the glucose found	,,		,,		58·80 ,,		117·60 ,,		,,	
Amount of carbon dioxide equivalent to the quantity of glucose added	,,		147·0 mg.		294·0 ,,		441·0 ,,		588·0 mg.	
Carbon dioxide unaccounted for	,,		58·67 ,,		119·61 ,,		183·70 ,,		394·50 ,,	
N/10 alkali required to neutralise the acidity	6·0 c.c.		6·5 c.c.		6·5 c.c.		7·0 c.c.		11·0 c.c.	

It will be observed from the table that the number of organisms does not fall although the carbon dioxide production decreases considerably after three or four days. It is even noticeable in most cases that the organisms have been multiplying after the carbon dioxide production has reached its minimum.

The experiments demonstrate that *B. prodigiosus* decomposes most readily glucose and laevulose, while sucrose comes next in order, lactose and maltose being very slightly decomposed. The amount of carbon dioxide evolved is not proportional to the amount of sugar added to the solution. In low concentrations the decomposition of the various sugars is more perfect than when the solutions are highly concentrated. Also the amount of carbon dioxide produced is not equivalent to the amount of sugar used up, which is not unexpected since various intermediate products are formed in addition to carbon dioxide. Organic acids are evidently one of these products as is shown by the figures for $N/10$ alkali required to neutralise the acidity in the various solutions after the experiment.

In order to find out as far as possible the various organic products produced by the action of *B. prodigiosus* on glucose, one litre of the nutrient solution containing 2 gm. of glucose was inoculated with the organisms and the amount of carbon dioxide estimated as in previous experiments. When the production of carbon dioxide had reached a minimum a qualitative organic analysis of the solution was carried out according to the method of J. R. Johnston(1). Alcohol, acetone and butyric acid were found to be definitely present, but such volatile compounds as formic and acetic acids were not found. Amongst the fixed acids tests for glycocholic, lactic, oxalic and succinic acids were made, but none could be definitely

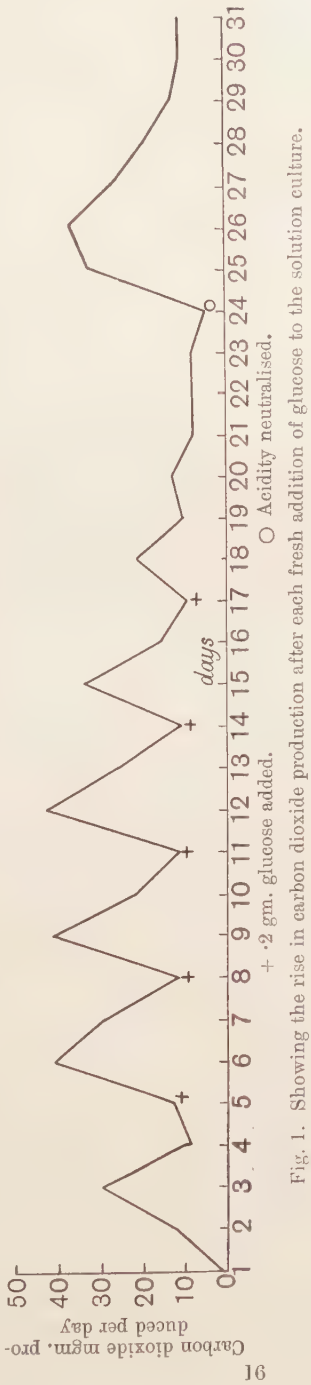
Carbon dioxide mgm. produced per day
0
10
20
30
40
50
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
days
+ ·2 gm. glucose added.
○ Acidity neutralised.

Fig. 1. Showing the rise in carbon dioxide production after each fresh addition of glucose to the solution culture.

proved to be present. The presence of a small quantity of a residue suggested, however, that some other fixed acids must have been produced.

The production of carbon dioxide always attains its highest value in the first three or four days and then falls off suddenly in spite of the presence of a sufficient quantity of sugar, and it is of interest that additions of fresh quantities of glucose, when carbon dioxide production has reached its minimum, rapidly induce further formation of carbon dioxide and the curve (Fig. 1) is practically identical after each addition of fresh material.

II. A Study of some of the Factors affecting the Carbon Dioxide Production in Soil.

While conducting experiments on the rate of carbon dioxide production in soils to which organic matter has been added, it was found that as in artificial cultures the carbon dioxide production reached its maximum in the first few days and then rapidly decreased. Some typical instances are given in Fig. 2. Probably a number of factors are responsible in bringing about this phenomenon, some of which are enumerated below:

Exhaustion of total carbon; exhaustion of readily available carbon; formation of an unattackable film on the surface of the particles of organic matter; exhaustion of some secondary food material, *e.g.* mineral nutrients necessary for the organisms; formation of toxic cleavage products; and inefficiency of the organisms.

The following experiments were therefore arranged to see if one or all of the above mentioned factors were responsible for lowering the carbon dioxide production.

(*a*) soil + 0·25 per cent. glucose + optimum moisture (16 per cent.). Nutrient solution containing all substances except carbon to be added when the carbon dioxide production decreased.

(*b*) soil + 0·25 per cent. starch + optimum moisture.

(*c*) soil + 0·25 per cent. glucose added in solution + optimum moisture. Further additions of 0·1 per cent. glucose to be made each time the carbon dioxide production decreased.

(*d*) soil + 0·25 per cent. glucose and nutrient solution (5 c.c. Omeliansky's solution) + optimum moisture.

(*e*) control; soil + optimum moisture.

(*f*) 100 c.c. ordinary bouillon solution inoculated with 1 gm. soil.

(*g*) 100 c.c. ordinary bouillon solution + 0·25 gm. glucose inoculated with 1 gm. soil.

The results of carbon dioxide production obtained from the above

experiment are given in Table VII, where the symbols refer to the treatment during the experiment; and the letters at the head of the columns to the initial soil treatments.

It will be seen from the results that exhaustion of total organic carbon does not appear to be a limiting factor since the cessation of

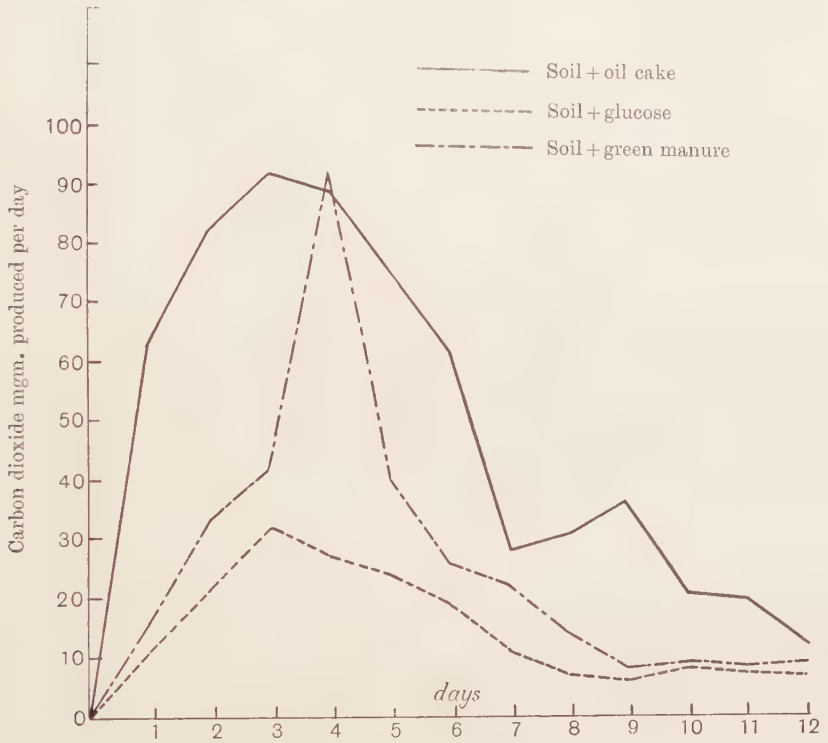

Fig. 2. Showing the general nature of carbon dioxide production in soil with oil cake, glucose or green manure.

carbon dioxide formation takes place even when there is an excess of carbon in the form of glucose present in the soil. This was found by qualitative tests of glucose in various experiments, the details of which are not recorded, and is in agreement with the results obtained in artificial culture solutions. Since the general trend of the carbon dioxide production curve as shown in Fig. 2 is practically similar whether complex organic matter like oil cake or green manure or simple chemical substances like glucose are used as a source of food material, it does not seem likely that the exhaustion of easily available carbon is responsible for the lowering of carbon dioxide production.

Table VII.

Showing milligrammes of carbon dioxide produced per day per 100 *gm. of soil in the case of* (*a*) *to* (*e*), *and per gm. of soil in* (*f*) *and* (*g*).

Days	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)	(*f*)	(*g*)
1	10·78	12·98	22·88	22·33	2·53	1·43	1·43
2	22·33	7·48	37·95	65·34	3·19	7·04	29·92
3	32·23	4·29	22·27	35·97	2·69	15·29	39·38
4	27·28	2·69	27·83	17·93	1·59	20·79	33·88
5	24·43	2·69	24·98	11·93	1·04	27·33	32·28
6	19·48	2·14	16·73	9·79	0·80	30·90	30·63
7	10·89	4·34*	11·00	6·54	1·34	27·88	27·33
8	7·20	6·54	7·86	5·99	1·45	10·28	21·50
9	6·10†	5·99	6·38	4·73	1·26	18·26	16·06
10	7·75	4·89	11·33‡	4·73§	1·26	12·21	14·74
11	7·15	4·18	22·11	4·73	1·04	7·49	15·73
12	6·93	3·63	19·69	4·18	1·26	9·68	17·16
13	5·61	4·18	11·66	3·63	1·26	12·37	17·71
14	5·28	4·40	7·48	3·63	1·26	9·51	18·81
15	4·73	4·73	6·38	3·30	1·48	9·68	20·46
16	3·35	3·46	5·83	3·19	0·60	10·23	22·22
17	3·46	3·46	5·39	2·86	1·26	10·56	23·76
18	3·24	3·24	5·06‖	2·36¶	1·26	10·12	24·31
19	—	5·66**	19·80	6·21	0·70	11·60	24·20
20	—	6·87	27·33	4·67	0·50	11·79	29·34
21	—	7·20	25·90	3·13	0·60	12·12	27·77
22	4·45††	8·52	31·07	2·91	0·70	17·40	29·42
23	3·90	7·97	24·14	2·14	0·50	10·58	28·43
24	3·35	7·86	21·39	—	0·90	10·58	29·20
25	4·67	6·87	16·61	—	0·70	10·14	26·67
26	2·14	6·87	15·40	—	0·90	8·60	22·27
27	1·95	5·00	11·00	—	0·40	8·38	14·30
28	1·73	5·22	9·35	—	0·50	3·98	11·55
29	1·29	4·67	7·97	—	0·30	7·42	8·52
30	1·54	4·18	5·83	—	0·40	7·26	8·03
31	1·43	4·18	5·28	—	0·40	6·38	9·68
32	—	4·18	4·73	—	0·50	6·93	6·93
33	—	4·18	5·28	—	0·50	7·81	6·38
34	—	4·18	—	—	0·50	6·38	6·93
35	—	3·63	—	—	0·90	6·60	5·50
36	—	3·08	—	—	0·50	6·82	6·38
37	—	3·30	—	—	0·50	1·98	5·28
38	—	5·08	—	—	0·50	6·93	4·73
39	—	2·64	—	—	0·80	9·68	4·73
40	—	2·42	—	—	0·80	6·60	5·28
41	—	—	—	—	0·80	5·83	5·28
42	—	—	—	—	0·70	4·73	3·63
43	—	—	—	—	0·70	5·28	3·63
44	—	—	—	—	0·80	6·38	4·73
45	—	—	—	—	0·80	6·60	3·63
46	—	—	—	—	0·80	5·28	3·63
47	—	—	—	—	—	5·17	3·08
48	—	—	—	—	—	4·84	2·86
49	—	—	—	—	—	4·84	2·64
50	—	—	—	—	—	3·74	2·64
51	—	—	—	—	—	3·74	2·42
52	—	—	—	—	—	3·19	2·42

* 0·05 gm. diastase added to the soil.
† Five c.c. Omeliansky's solution added.
‡ 0·2 gm. glucose added in solution.
§ Five c.c. 2 % ammonium sulphate solution added.
‖ Added 0·5 gm. glucose in solution.
¶ Heated in the steamer for half an hour.
** Inoculated the soil with *Aspergillus* sp.
†† Soil left unaspirated for 3 days and then started again.

Addition of a complete mineral solution when the carbon dioxide production has fallen has a slight but insignificant stimulating effect; as shown in Exp. (*a*), 9th day; nor does the addition of ammonium sulphate solution stimulate the process ((*d*), 10th day).

The curve of carbon dioxide production is practically identical whether the organic matter is added to the soil or solution cultures and whether the organic matter used is of a complex chemical and mostly insoluble nature like oil cake or of a comparatively simple and soluble nature like glucose, Exps. (*a*) and (*g*). This suggests that the formation of an unattackable film round the particles of the organic matter is not an important factor concerned in lowering the rate of carbon dioxide production.

Successive additions of glucose to soil (see Exp. (*c*), 10th and 18th days), or to the solution (Fig. 1), when the carbon dioxide production has reached its minimum, increased this process to the same level as in the beginning. Similar results were obtained when oil cake instead of glucose was used as a source of organic matter added to the soil.

That the lowering of the carbon dioxide production is not due to the inefficiency or reduction in the number of organisms has already been shown (Table VI). The fact that fresh additions of glucose to soil increase the carbon dioxide production also shows that the organisms present in the soil have not lost their efficiency.

Having observed the above facts it was decided to carry out further experiments to find out if possible any interference in the process of carbon dioxide formation due to the production of toxic substances. If we consider that toxic substances are destroyed by various mechanical treatments such as heat, sunlight, aeration, etc., as suggested by Grieg Smith (2), then an indirect method of testing the presence of toxic substances would be to test the effects of such mechanical treatments on the assumption that they would destroy the toxic substances and thus stimulate the process of carbon dioxide formation for a longer period.

The following experiments were therefore carried out:

200 gm. of soil of 16 per cent. moisture content and containing 0·25 per cent. of glucose were treated in the following manner:

(*a*) Soil stirred when carbon dioxide production showed signs of decrease.

(*b*) Soil partially sterilised when carbon dioxide production showed signs of decrease.

(*c*) Soil washed when carbon dioxide production showed signs of decrease.

(*d*) Control without any treatment.

The results obtained are given below (Table VIII), where it will be seen that none of the mechanical treatments employed are of any use in stimulating the process of carbon dioxide production when once it has begun to decline.

Table VIII.

Showing milligrammes of carbon dioxide produced per 100 gm. of soil per day.

Days	Soil stirred (*a*)	Soil sterilised (*b*)	Soil washed (*c*)	Untreated (*d*)
1	10·78	22·88	22·55	22·11
2	24·47	31·62	33·27	33·27
3	16·99	17·87	16·44	17·65
4	12·37*	11·27†	9·90‡	10·45
5	9·90	6·05	—	8·80
6	5·63	4·53	—	—
7	5·17	4·84	5·17	5·17
8	4·62	2·97	4·62	4·62
9	6·38	2·53	3·30	4·18
10	7·48	2·20	3·08	3·63
11	3·63	1·43	3·08	3·08
12	3·96	1·43	3·08	2·53
13	3·08	1·43	3·08	2·48
14	3·08	1·98	2·53	2·48
15	3·19	0·99	2·09	1·54
16	1·54	1·21	1·54	2·09
17	3·19	1·76	1·21	2·31
Total	126·46	116·99	114·94	126·39

* Stirred the soil for five minutes.

† Heated the soil in the steamer for half an hour.

‡ Washed the soil with 600 c.c. of water and started again when the moisture had gone down to 16 %.

The question of the formation of toxic cleavage products and their effect on the process of carbon dioxide production does not appear to be at all clear and, in the opinion of the writer, more work in this direction by both direct and indirect methods should prove very valuable in solving this problem.

The above experiments were carried out some years ago by the writer as a post-graduate student at the Imperial Agricultural Research Institute, Pusa (India), and they are by no means complete. Since the author has had no further opportunity to continue this piece of work it was considered desirable to place on record the results so far obtained with the hope that they would be of some use to other workers working on similar problems.

In conclusion it is with great pleasure that I acknowledge my indebtedness to Mr D. W. Cutler, M.A., for his inspiration and kind help in the preparation of this paper.

Summary.

(1) The rate of carbon dioxide production by *B. prodigiosus* from various sugars has been studied and an account of experiments conducted with a view to finding out the factor or factors responsible in lowering the carbon dioxide production in soil and solution is given.

(2) It is found that *B. prodigiosus* can decompose glucose and laevulose most readily while sucrose comes next in order. Lactose and maltose are very slightly decomposed.

(3) The amount of carbon dioxide produced is not equivalent to the amount of sugar used up. Other organic products like alcohol, acetone and organic acids are produced.

(4) Carbon dioxide production always attains its highest limit within the first three or four days and then declines rapidly in spite of the presence of sugar and active organisms.

(5) Successive additions of fresh quantities of sugar to solution cultures when the carbon dioxide production has reached its minimum rapidly increase the carbon dioxide formation to a value practically identical with that previously attained.

(6) It appears that exhaustion of total or readily available carbon, formation of an unattackable film on the surface of particles of organic matter, or exhaustion of available mineral constituents is not responsible for lowering the carbon dioxide production in soil.

(7) Additions of organic matter such as glucose or oil cake to soil after the carbon dioxide production has declined, restores the process practically to the same level as at the start.

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(1) Johnston, J. R. (1912). *U.S. Dept. Agri. Bur. Plant Industry Bull.* No. 228.

(2) Grieg Smith, R. (1910, 1913). *Proc. Linn. Soc. N.S.W.* xxxv, 808–822, xxxviii, 725–746.

(*Received December 9th*, 1925.)

THE EFFECT OF SOME SOIL CONDITIONS ON NODULE FORMATION ON *CROTALARIA JUNCEA* (L.)[1]

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(With 1 Diagram.)

Introductory.

Whilst the wide distribution of leguminous crops in India under varying conditions suggests that organisms known to form nodules on the roots of such plants may be extremely adaptable to their environment, marked differences in their growth in different parts of the country indicate, among other factors, how dominant must be the influence of soil and climatic conditions on the activities of the causal organism of nodule production (*B. radicicola*).

The effect of some of the elements essential for plant growth on the formation and growth of nodules on the roots of leguminous crops has been studied by a number of investigators. Prucha (1) found that an application of phosphorus, calcium or potassium appreciably increased the number of nodules. Wilson (2), using various chemical substances in soil or water culture, observed that phosphates and calcium compounds were effective in stimulating nodule formation. Fellers' experiments (3) also indicate that the chemical factors introduced by the addition of phosphorus, calcium or potassium, influence nodule formation.

In experiments with some of the important leguminous crops grown in India, the writer (4) has shown that phosphorus, potash and lime, when applied to the black cotton soil, materially increase the formation and development of nodules. However, a number of preliminary tests with various types of soils gave contradictory results; and suggested that a study of the factors, such as soil texture, soil reaction, soil moisture and soil aeration might have important relations to nodule formation. Relatively little work has been done to determine the influence of such factors. Wilson (2) found that "as the moisture was increased from 25 per

[1] This investigation was carried out in the Department of Bacteriology, Rothamsted Experimental Station, Harpenden.

cent. of the dry soil to 75 per cent., the nodule formation was correspondingly increased." Bryan(5), experimenting with a number of leguminous crops to determine the effect of reaction on growth and nodule formation, came to the conclusion that "nodules form at any reaction at which the plants grow, but the greatest number are formed at or near neutrality."

Scope.

In view of the fact that soil texture, soil moisture and soil reaction may be largely interdependent, it is necessary to study their collective as well as their individual influence in stimulating nodule formation on a leguminous plant, keeping all other conditions of growth controlled as much as possible under greenhouse conditions. The work reported in this paper was carried out in the Rothamsted Experimental Station with the object of studying to what extent each of the above factors behave collectively and individually.

The plant chosen was *Crotalaria juncea* (sunn hemp), a most commonly cultivated Indian leguminous crop. The experiment was outlined by Mr H. J. Page, and many helpful suggestions in the interpretation of the results came from Mr H. G. Thornton. The writer takes pleasure in expressing his gratitude to them, and to many others who assisted him in carrying out the experiment.

The writer is greatly indebted to Mr R. A. Fisher for his kindness in considering the data from the statistical point of view.

Methods.

Sixty glazed cylindrical pots of 22·5 lbs. capacity were filled with soil passed through a 3 mm. sieve; or with soil-sand mixtures with varying percentages of coarse sand, one group receiving no sand, one 20 per cent. sand, and the last 40 per cent. sand. The soil in these pots was obtained from the Harpenden Common, and six additional pots were set up with garden soil. The mechanical analysis of the Harpenden Common soil is given below:

	%
Fine sand	18·86
Silt	34·62
Fine silt I	4·99
„ II	6·14
Clay	19·76
Loss on ignition	10·12

A basal dressing of 3·6 gm. of superphosphate and 0·72 gm. of sulphate of potash was given to each pot in every series.

Soil moisture was varied in proportion to the water-holding capacity and wilting coefficient ascertained for each type of soil and soil-sand mixture used in the experiment. On this basis, three degrees of moisture were tested in each series. Thus, the moisture for series A (see Table I) with 64 per cent. water-holding capacity, was kept constant to the limits of 15 per cent. as minimum, 25 per cent. as medium, and 40 per cent. as maximum; for series B with 48 per cent. water-holding capacity, the limits were 10 per cent., 20 per cent. and 30 per cent.; and for series C with 34 per cent. water-holding capacity, 7·5 per cent., 12 per cent. and 20 per cent. The moisture content of series D with 32·5 per cent. water-holding capacity, was kept at 10 per cent. as minimum, 15 per cent. as medium and 30 per cent. as maximum. The range of calculated percentage of moisture was kept constant by weighing the pots every week and making up the loss as required.

The *p*H value of the Harpenden Common soil was determined by the electrometric method, which indicated 5·03. The varying alkalinity was obtained by adding calcium carbonate on the basis of lime requirement, which was 0·43 per cent. Three different soil reactions were tested, namely, acid (5·03), neutral (7·05) and alkaline (8·00). After the addition of the required $CaCO_3$, the *p*H values were determined by the colorimetric method in order to be sure that the range of soil reaction was obtained. Thus, the effect of nine variations in soil conditions were tested, namely, three degrees of texture, three degrees of moisture and three degrees of soil reaction.

A table showing the plan of the experiment is given below.

Table I.

Plan of the Experiment.

	A Harpenden common soil			B Common soil +20 % sand			C Common soil +40 % sand			D Garden soil		
Moisture	Min. 15 %	Med. 25 %	Max. 40 %	Min. 10 %	Med. 20 %	Max. 30 %	Min. 7·5 %	Med. 12 %	Max. 20 %	Min. 10 %	Med. 15 %	Max. 30 %
Acid	1 A	3 A	5 A	1 B	3 B	5 B	1 C	3 C	5 C	—	—	—
*p*H 5·03	2 A	4 A	6 A	2 B	4 B	6 B	2 C	4 C	6 C	—	—	—
Neutral	7 A	9 A	11 A	7 B	9 B	11 B	7 C	9 C	11 C	1 D	3 D	5 D
*p*H 7·05	8 A	10 A	12 A	8 B	10 B	12 B	8 C	10 C	12 C	2 D	4 D	6 D
Alkaline	13 A	15 A	17 A	13 B	15 B	17 B	13 C	15 C	17 C	—	—	—
*p*H 8·00	14 A	16 A	18 A	14 B	16 B	18 B	14 C	16 C	18 C	—	—	—

Inoculation was accomplished by using a suspension obtained from a sample of Bengal soil sent to the writer by the Director of Agriculture, Bengal, from a field where *Crotalaria juncea* was recently grown. Each pot received the same quantity of inoculum.

To minimise the effect of varying seed weights, ten seeds of approximate uniform weights were sown in each pot. Each pot was thinned out

Table II.

Showing number of nodules after the fourth week from the date of inoculation.

Pot number	Treatment			Number of nodules per four plants	Average weight of air-dry tops
	Texture	Soil reaction	Soil moisture		
1 A	Harpenden Common soil	Acid	Min.	5	0·48
3 A	,,	,,	Med.	9	0·69
5 A	,,	,,	Max.	14	0·79
7 A	,,	Neutral	Min.	10	0·51
9 A	,,	,,	Med.	17	0·95
11 A	,,	,,	Max.	19	1·04
13 A	,,	Alkaline	Min.	11	0·53
15 A	,,	,,	Med.	16	0·95
17 A	,,	,,	Max.	25	1·45
1 B	Harpenden Common soil +20 % sand	Acid	Min.	3	0·59
3 B	,,	,,	Med.	13	0·94
5 B	,,	,,	Max.	16	1·11
7 B	,,	Neutral	Min.	11	1·01
9 B	,,	,,	Med.	9	1·19
11 B	,,	,,	Max.	17	1·05
13 B	,,	Alkaline	Min.	11	0·63
15 B	,,	,,	Med.	14	0·66
17 B	,,	,,	Max.	21	1·15
1 C	Harpenden Common soil +40 % sand	Acid	Min.	4	0·55
3 C	,,	,,	Med.	7	0·69
5 C	,,	,,	Max.	12	0·97
7 C	,,	Neutral	Min.	8	0·95
9 C	,,	,,	Med.	13	1·09
11 C	,,	,,	Max.	13	1·18
13 C	,,	Alkaline	Min.	11	0·96
15 C	,,	,,	Med.	12	1·23
17 C	,,	,,	Max.	19	1·27
1 D	Garden soil	Neutral	Min.	11	1·22
3 D	,,	,,	Med.	21	1·87
5 D	,,	,,	Max.	39	2·31

Table III.

Showing the number of nodules after the sixteenth week from the date of inoculation.

Pot number	Treatment: Texture	Treatment: Soil reaction	Treatment: Soil moisture	Number of nodules per four plants	Average weight of air-dry tops
2 A	Harpenden Common soil	Acid	Min.	6	1·81
4 A	,,	,,	Med.	11	2·12
6 A	,,	,,	Max.	29	3·05
8 A	,,	Neutral	Min.	11	1·44
10 A	,,	,,	Med.	23	2·71
12 A	,,	,,	Max.	36	3·17
14 A	,,	Alkaline	Min.	19	1·95
16 A	,,	,,	Med.	27	2·58
18 A	,,	,,	Max.	43	4·12
2 B	Harpenden Common soil +20 % sand	Acid	Min.	4	1·12
4 B	,,	,,	Med.	17	2·00
6 B	,,	,,	Max.	21	2·22
8 B	,,	Neutral	Min.	13	2·01
10 B	,,	,,	Med.	25	3·16
12 B	,,	,,	Max.	33	3·89
14 B	,,	Alkaline	Min.	24	1·54
16 B	,,	,,	Med.	31	2·57
18 B	,,	,,	Max.	48	4·05
2 C	Harpenden Common soil +40 % sand	Acid	Min.	7	1·01
4 C	,,	,,	Med.	22	2·00
6 C	,,	,,	Max.	39	3·11
8 C	,,	Neutral	Min.	17	1·93
10 C	,,	,,	Med.	36	2·51
12 C	,,	,,	Max.	41	3·29
14 C	,,	Alkaline	Min.	19	2·48
16 C	,,	,,	Med.	39	3·12
18 C	,,	,,	Max.	67	4·65
2 D	Garden soil	Neutral	Min.	35	4·85
4 D	,,	,,	Med.	57	5·21
6 D	,,	,,	Max.	92	7·05

to six plants after proper germination but before inoculum and finally only four plants were left in each pot. At the end of 8 weeks of growth, or after the 4th week from the date of inoculation, the soil from one pot in each series was carefully washed out with a hose, and the roots were freed from soil particles by carefully washing them in water. The remaining pots were similarly treated at the end of the experiment, 16 weeks

after inoculation. The nodules were counted with the help of a lens by placing the plants against a black surface.

Nitrogen of tops of the plants at harvest was determined by the Kjeldahl method.

RESULTS.

Crotalaria juncea is a tropical plant. Under greenhouse conditions it did not grow to its usual height. The plants in the pots treated with maximum water were in partial or full bloom, those in the D series reaching the height of about 23 in.

The plants examined before inoculation were found entirely free from nodules on their roots. The numbers of nodules and average weight of air-dry tops of the plants after the 4th week from the date of inoculation are given in Table II. Similar data at the time of harvesting the plants

Table IV.

Analysis of plants at harvest.

	Treatment				
Pot number	Soil texture	Soil moisture	Soil reaction	% dry matter	% nitrogen
2 A	Harpenden Common soil	Min.	Acid	91·90	3·36
6 A	,,	Max.	,,	89·80	3·21
8 A	,,	Min.	Neutral	88·90	2·84
12 A	,,	Max.	,,	88·85	2·90
14 A	,,	Min.	Alkaline	89·05	3·76
18 A	,,	Max.	,,	89·10	2·87
2 B	Harpenden Common soil +20 % sand	Min.	Acid	86·90	3·36
6 B	,,	Max.	,,	88·30	3·03
8 B	,,	Min.	Neutral	89·40	3·15
12 B	,,	Max.	,,	88·80	2·84
14 B	,,	Min.	Alkaline	90·90	3·29
18 B	,,	Max.	,,	88·90	3·55
2 C	Harpenden Common soil +40 % sand	Min.	Acid	90·20	3·50
6 C	,,	Max.	,,	89·10	2·83
8 C	,,	Min.	Neutral	89·30	2·93
12 C	,,	Max.	,,	90·35	3·22
14 C	,,	Min.	Alkaline	90·80	3·09
18 C	,,	Max.	,,	89·10	3·14
2 D	Garden soil	Min.	Neutral	88·40	2·78
6 D	,,	Max.	,,	88·60	2·79

The writer is indebted to Mr G. C. Sawyer for the analysis recorded in this table.

are given in Table III, and percentages of nitrogen and dry matter of the plants from minimum and maximum moisture pots of each series are shown in Table IV.

Discussion of the Results.

To what extent soil moisture influences nodule formation may be seen from Table V, in which the total number of nodules produced under varying degrees of moisture with three different soil reactions are given. In each case the effect of moisture is noticeable from the first stage of infection, the number of nodules increasing with higher degree of saturation. It tends to show that the water content probably plays a significant rôle in giving a better opportunity to the organisms for infection.

Table V.

Showing number of nodules under varying soil-moisture.

	4th week count				16th week count			
	Dry	Med.	Wet	Total	Dry	Med.	Wet	Total
Acid	12	29	42	83	17	50	89	156
Neutral	29	39	49	117	41	84	110	235
Alkaline	33	42	65	140	62	97	158	317
Total	74	110	156	340	120	231	357	708

Table VI.

Showing number of nodules under varying soil-texture.

	4th week count				16th week count			
	Heavy	Med.	Light	Total	Heavy	Med.	Light	Total
Acid	28	32	23	83	46	42	68	156
Neutral	46	37	34	117	70	71	94	235
Alkaline	52	46	42	140	89	103	125	317
Total	126	115	99	340	205	216	287	708

The addition of sand seems to have a marked effect on the number of nodules formed. In Table VI it is shown that in every case the series with 40 per cent. sand gave the largest number of nodules, although the 4th week count recorded in favour of soil alone. In explanation of this result, it is suggested that in the heavier soil medium a dense population of organisms near the point of infection favours the infection of the young roots, while, in the lighter mixtures, the organisms become more sparsely distributed through a wider volume of soil so that the chances of infection on the seedling roots are diminished but the formation

of nodules on the more remote parts of the mature root system is rendered possible.

It can be seen in Tables V and VI that alkalinity is more favourable for nodule production than neutral reaction. This effect is noticeable in each series from the 4th week to the harvesting time. The relation of soil texture, moisture and soil reaction is shown graphically in Diagram 1.

It is interesting to note that in the garden soil, with a high degree of moisture content, the number of nodules rose as high as 92 per four

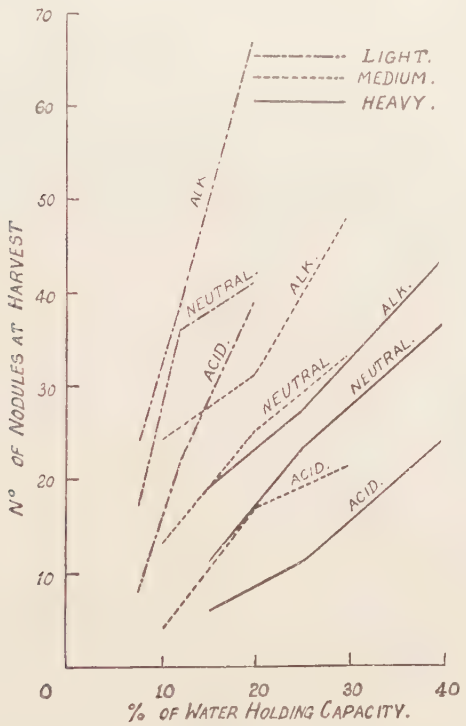

Diagram 1.

plants, in contrast with 43 in the case of those grown in the Harpenden Common Soil. Although no definite conclusion may be reached from such scanty data, the result indicates that the complex constituents of garden soil may furnish the organisms with some easily available sources of energy; or the plant itself may have been directly benefited by the rich soil.

There seems to be no definite correlation between the weight of air-dry tops and the number of nodules; nor do the latter bear any relation to the nitrogen figures obtained.

Finally, the experiments show that, in view of the results of the statistical interpretation (see Appendix) three sets of factors have acted quite independently of one another. Apart from their influence on the plant growth, the study of each of these factors on the nodule-forming organism is, therefore, important.

Summary.

From pot culture experiments in the greenhouse it has been shown that the formation of nodules on the roots of *Crotalaria juncea* is affected significantly by variations in the texture, moisture content and reaction of the soil, all other conditions being kept uniform. Nodule formation was increased by higher moisture content, by increased coarseness and by reduced hydrogen ion concentration.

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APPENDIX

STATISTICAL INTERPRETATION OF TABLES I AND II OF THE ABOVE PAPER

By R. A. FISHER, Sc.D.

(With 2 Diagrams.)

There are theoretical reasons for anticipating that equal variability will be shown, not by the actual nodule numbers but by their square roots; it is not improbable also that equal changes in the three variates investigated, *i.e.* soil texture, soil reaction, and soil moisture, may affect the square roots equally, and that these effects may be independent. These anticipations turned out to be warranted by the data tabulated in Tables I and II of the above paper. The square roots of the observed numbers were therefore fitted by a prediction formula

$$m + ai + bj + ck,$$

where m is the expected value for medium texture, *i.e.* soil containing 20 per cent. sand, neutral reaction and medium water content; i, j and k take the values -1, 0, 1, giving 27 combinations corresponding to the 27 experiments, and a, b and c represent respectively the effects of modifying the texture, the reaction and the moisture. The values found were

		4th week	16th week
	m	3·468	4·916
Texture	a	0·203	0·418
Reaction	b	0·482	0·937
Moisture	c	0·667	1·349

The standard errors of these latter quantities are all equal and are found from the sum of the squares of the residuals; divided by 23, the number of degrees of freedom left after fitting the above four quantities; this gives the variance of individual observations; this again divided by 18 gives the variance of a, b or c. The standard errors derived in this way 0·0750 for the 4th week and 0·0963 for the 16th week nodule counts; and it is evident that at both dates all three of the quantities evaluated are statistically significant.

By squaring the values of the prediction formula we may obtain the theoretical numbers of nodules to be expected in each experiment. The close agreement of expectation with the observed numbers is shown in

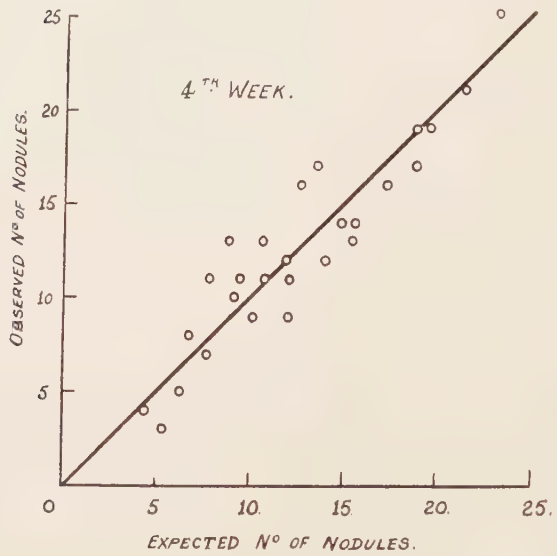

Diagram 2.

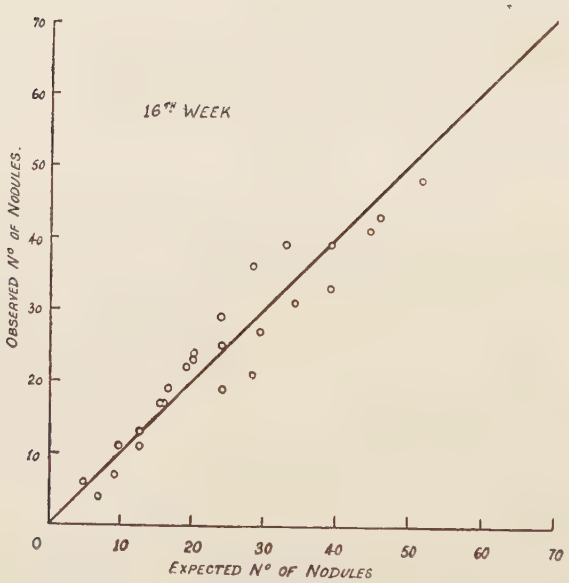

Diagram 3.

the two Diagrams 2 and 3, one representing the data obtained from the 4th week, and the other at the end of the experiment, which is the 16th week. The actual numbers of nodules and prediction values are given in Table VII.

Table VII.

Observations on the 4th week		Observations on the 16th week	
Actual number observed	Prediction value	Actual number observed	Prediction value
5	6·36	6	4·89
9	10·17	11	12·68
14	14·87	29	24·11
10	9·02	11	9·92
17	13·48	23	20·23
19	18·83	36	34·17
11	12·15	19	16·70
16	17·24	27	29·52
25	23·06	43	46·01
3	5·38	4	6·92
13	8·92	17	15·83
16	12·70	21	28·39
11	7·85	13	12·72
9	12·03	25	24·17
17	18·81	33	39·24
11	10·78	24	30·29
14	15·60	31	34·24
21	21·31	48	51·85
4	4·48	7	9·29
7	7·75	22	19·33
12	11·91	39	33·01
8	6·75	17	15·88
13	10·66	36	28·45
13	15·46	41	44·65
11	9·49	19	24·23
12	14·04	39	39·31
19	19·49	67	58·04

(*Received September 30th*, 1925.)

THE ORGANISM FORMING NODULES ON *CROTALARIA JUNCEA* (L.)[1]

By N. GANGULEE, Ph.D.

(*Khaira Professor of Agriculture, University of Calcutta.*)

Introduction.

Failure to induce nodule formation when *Crotalaria* (sunn hemp) was grown in Rothamsted garden soil with unsterilised seed (4) led to the idea of effecting inoculation with organisms from (*a*) bean, (*b*) lucerne, and (*c*) soy bean nodules. Suspensions of pure cultures made in nitrogen-free salt solution were used in inoculating the sterile soil, but no nodules were formed for 4 weeks, although the plants attained reasonable growth. Nodules were formed only after inoculating with a suspension made from the soil where *Crotalaria* had previously grown. This confirms the conclusion arrived at by various investigators that the nodule-forming organisms of the legumes do belong to different physiological varieties. Having obtained satisfactory infection, the organism was isolated from the nodules in order to investigate its morphology.

The work reported in this paper concerns the changes through which the organism passes when grown on different media.

Method of Isolation. In isolating the organism from *Crotalaria* nodules the method suggested by Harrison and Barlow (1) was used. A medium-sized nodule, after soil particles had been washed off, was immersed for two minutes in mercuric chloride solution, and washed in sterile distilled water. A small portion of the tissue inside the nodule was dug out aseptically and crushed in sterile water. A loopful of this suspension was plated on soil extract bean agar. Only one type of organism appeared and the culture was transferred to agar slopes for study of its morphology and for its growth on the range of media indicated below.

Growth on agar media. The following agar media were used:

1. Soil extract agar.
2. Soil extract saccharose agar.

[1] This investigation was carried out in the Department of Bacteriology, Rothamsted Experimental Station, Harpenden.

3. Soil extract mannite agar.
4. Soil extract bean agar.
5. Bean extract agar.
6. Prucha medium.

The soil extract was made by steaming one kilo of garden soil with two litres of distilled water for a period of 2 hours, and passing the cooled supernatant liquid through a Berkfeld filter candle.

The bean seedling extract was prepared as follows: The bean seedlings were pounded up in a mortar, covered with water in a large flask, and boiled for an hour. After standing for 24 hours the extract was filtered and diluted to give 2 gm. of dry matter to 1000 c.c. of water. To 1000 c.c. of bean extract were added:

Saccharose	10	gm.
Asparagine	0·75	,,
Di-potassium phosphate	0·1	,,
Calcium carbonate	5·0	,,
Agar	20·0	,,

Ashby solution (2) was used with soil extract, soil extract saccharose and soil extract mannite agar.

Morphology. The organism appears to pass through the life cycle described by Bewley and Hutchinson (3). A 3 days old culture shows motile rod-shaped organisms with more than one flagellum. In fresh cultures cocci and short rods predominate, but in older cultures banded rods appear. After a week's growth on soil extract saccharose (or mannite) agar the rods measure 3–4 μ long and about 1·5 μ broad. No branched forms were seen.

The organism stains well with carbol-fuchsin. For staining flagella the method employed has been that devised by Mr P. H. Gray (5), to whom the writer desires to express his thanks.

Agar colonies. The colonies which appear on soil extract saccharose agar incubated at 25° C. for a week are quite uniform and characteristic of this nodule organism. The surface colonies at 3 days are 1 mm. round, raised greyish white, glistening, smooth with edges entire; deep colonies smaller, whitish, lens-shaped.

Growth on sloped agar media. In making sub-cultures on the agar slopes mentioned below a 14 days old culture was used.

The cultures were incubated at 25° C. Although the organism grows well on a great variety of culture media the rate of growth varies with their composition. The results of observations made at various intervals

on different media are given below. The predominant forms were determined by examining the slides prepared at the time of observation. "Growth" at 30 days refers to the appearance of the growth in the water collected at the base of the agar slope.

Table I.

Relative amount and appearance of Growth and Predominant Forms.

Media	3 days		7 days	
	Growth	Form	Growth	Form
1. Soil extract agar	Slight	Cocci and short rods	Moderate	Short and banded rods; few motile
2. Soil extract saccharose agar	Good	Cocci and short rods; motile	Very good	Short rods
3. Soil extract mannite agar	Good	Cocci and short rods; motile	Good	Short rods and cocci
4. Soil extract bean agar	Moderate	Cocci and short rods; motile	Moderate	Short rods and cocci; few motile
5. Bean extract	Slight	Cocci and short rods	Moderate	Banded rods and cocci; few motile
6. Prucha medium	Very slight	Banded rods; few short rods	Poor	Banded rods; few short* rods

Media	14 days		30 days	
	Growth	Form	Growth	Form
1. Soil extract agar	Good	Banded rods	Clear	Banded rods
2. Soil extract saccharose agar	Very good	Banded rods; some short rods	Thick mucilaginous	Banded rods; some cocci
3. Soil extract mannite agar	Very good	Banded rods; some short rods	Thick mucilaginous	Banded rods; some cocci
4. Soil extract bean agar	Good	Banded rods; some short rods	Less mucilaginous	Banded rods; some cocci
5. Bean extract	Moderate	Banded rods	Clear	Banded rods
6. Prucha medium	Poor	Banded rods; few short rods	Clear	Banded rods

* A few motile forms in condensation water.

Of the solid media used, soil extract agar with saccharose, mannite, or bean seedling decoction induced rich growth. Among the rest, in Prucha medium the growth was relatively poor. The presence of sugars, such as saccharose or mannite, tends to produce in 1 month a thick mucilaginous growth in the condensation water. The time taken before the rapid growth commenced has been also much shorter in these media; and it is interesting to note that the quick growth is correlated with the early appearance of motile rods.

Growth on liquid media. Four flasks containing 50 c.c. of the sterile media as indicated below were inoculated with two drops of bacterial

suspension made by emulsifying a 14 days old soil-extract saccharose agar slope culture with 10 c.c. of sterile water, and incubated at 25° C. At intervals of 3, 7, 15 and 30 days, a loopful from each of the flasks was examined under the microscope. After 3 days each flask showed turbidity and, except in the case of distilled water, a flocculent precipitate settled at the end of the month.

Table II.

Predominant forms.

Media	3 days	7 days	15 days	30 days
Distilled water	Short rods	Short rods and cocci	A few banded rods	None visible
Ashby solution	Short rods and cocci	Short rods and cocci; motile	Banded and short rods	Banded and short rods
Bean extract	Short rods	Short rods and cocci; a few motile	Banded rods; some short rods	Banded and short rods
Soil extract	Short rods; motile	Short rods and cocci; motile	Banded rods; some short rods	Banded and short rods

Of the liquid media used only soil extract had the effect of inducing motility within 3 days. In comparing the liquid with the solid media it is to be noted that soil extract can replace saccharose in bean agar, in encouraging early motility. Ashby solution, on the other hand, when added to soil extract agar, had the effect of retarding it.

CONCLUSION.

The observations recorded in this paper show that the stages which have previously been known to take place in the life cycle of *B. radicicola* from certain leguminous plants, occur also in the life cycle of the organism isolated from nodules of *Crotalaria juncea*. These changes occur both in solid and in liquid media. The coccoid bodies have been observed both in the non-motile ("Preswarmer") and the motile ("Swarmer") stages. These are succeeded by the short-rod stage, which eventually gives rise to the banded (vacuolated) forms. The outset and duration of these stages varies with the composition of the media.

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(*Received September 30th*, 1925.)

AN INTRODUCTORY NOTE ON TWO BACTERIA CAUSING AN INTERNAL ROT OF COTTON BOLLS

By J. C. HOPKINS.

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The two bacteria were isolated from "Durango" bolls growing at the experiment station, St Augustine, Trinidad. The seed had been imported from the Bureau of Plant Industry and was being tried out on the experiment plots together with five other varieties of cotton, so that this was the first generation which had been grown under Trinidad conditions. It would be presumptuous to classify this plant affection as a serious disease, since its prevalence has not been established, but it is worthy of note that both bacteria are capable of producing an internal boll rot and one of the organisms appears to be associated with anthracnose.

It will be convenient to consider the two organisms together, as the majority of the experiments carried out were identical for each; a detailed description is given later. The serial number will be used to distinguish one from the other. Thus O. 1 is a yellow organism and was isolated from a dark spot with a whitish centre. This also yielded a species of *Colletotrichum*. Y. 3 was obtained from a small water-soaked area near the stalk end of the boll and no other organism was isolated.

The method of isolation was in all cases the same. The surface of the boll was washed with soap and water, dipped for 30 seconds into 0·1 per cent. aqueous solution of mercuric chloride and then washed for 1 minute in sterile water. In the original isolation the outer tissues of the boll wall were removed by means of a sterile scalpel and a small piece of the inner tissues teased out in nutrient broth (Difco) and left for 20 minutes. Plates of three dilutions in nutrient agar (Difco + 10 Fuller) + 0·5 per cent. saccharose were thrown and slopes made from single colonies after 24 and 36 hours, according to growth. In the case of isolation from the internal rot, small pieces of the seed coat were substituted for the boll wall tissue.

Trial experiments were made with needle-prick inoculations on cut bolls resting with their stalks in sterile water in small bottles, under a

bell-jar which had been wiped with mercuric chloride solution, and it was found that the organism did not spread through the boll wall under these conditions, and no internal rot was brought about. Similar results were obtained by spraying a suspension of the bacteria on uninjured bolls. As a result, injections were made by means of a hypodermic syringe into the centre of unpicked, young, growing bolls. The surface of the boll was wiped with 0·1 per cent. mercuric chloride solution for 30 seconds and then wiped with absolute alcohol. The bacterial suspension was injected and the puncture covered with sterile vaseline. Controls were used in which sterile water alone was injected, under identical conditions. All the plants were grown in pots in a glasshouse and shaded from the sun by white muslin.

Five varieties of cotton[1] (Sea Island, Durango, Acala, Lone Star and Pima) were experimented with and in each case an internal rot was brought about by both organisms, re-isolations being made from infected tissue; these cultures again produced the diseased condition when inoculated into healthy bolls. In each case where infection was brought about, the organism was isolated and its pathogenicity established by successful infection of healthy bolls. In all experiments plates were thrown from tissues of the control bolls and one plate alone contained a single yellow colony which did not correspond to either O. 1 or Y. 3.

The rots produced by the two bacteria are quite similar in character. In 4 days the seed coat is attacked and turns brown, later the lint becomes discoloured and the internal tissues of the boll wall present a water-soaked appearance, which in some cases may extend to the exterior. Later still the endosperm and cotyledons of the seed are attacked and rotted, the whole of the contents of the loculus becoming a soft pulpy mass. The central placenta may be discoloured, but an extensive rot of the harder tissues does not appear to take place. The rot does not seem capable of extending beyond the loculus into which the bacterial suspension has been injected, for even after as long a period as three weeks, no staining was observed in any other loculus. Control bolls examined after this length of time showed a pale water-soaking of the lint in the

[1]

Commercial name	Group	Systematic name
Cauto	Brazilian	*Gossypium braziliense* var. *apospermum*
Pima	Egyptian	*G. peruvianum* var.
Sea Island	Sea Island	*G. barbadense* var. *maritimum*
Durango, Acala, Lone Star, Meade	American upland	*G. hirsutum* vars.

inoculated loculus, but the immature seed remained white and the presence of no pathogenic organism could be detected.

Microscopic Examination. Sections of newly diseased seeds showed bacteria in the outer tissues of the testa in great abundance and the organism could be seen on the exterior of the lint hairs, but in no case were bacteria observed in the lumina. In bolls which had been infected for a longer period, up to 10 days, the organism had penetrated into the interior of the seed, but although the lint had become discoloured, the lumina of the hair appeared to be free from bacteria. It was only in bolls in which the contents of the infected loculus had become completely disorganised and the lint hairs killed as a result of the rotting of the seed tissues, that bacteria were observed *in* the lumina. Sections of the boll wall in the region of the water-soaking showed, for O. 1, the presence of bacteria in the intercellular spaces, which were filled with liquid, and nutrient agar plates thrown from this tissue gave colonies of O. 1. This, however, was only observed in two "Durango" bolls. The water-soaking of the boll wall tissue was not a common occurrence but was undoubtedly due to the inoculated organism. Y. 3 did not produce any water-soaking of the boll wall externally, although in some cases the inner tissues attained this condition.

Association with Anthracnose Disease. As already mentioned, a species of *Colletotrichum* was isolated from the diseased tissue which yielded O. 1. Needle-prick inoculations with O. 1 having failed to bring about infection, experiments were done with a combination of both bacterium and fungus.

Bolls of the following varieties of cotton, Sea Island, Durango, Lone Star, Acala, Meade and Cauto were sterilised with mercuric chloride solution as before, and placed in sterile damp dishes. Multiple needle-prick inoculations with O. 1 were made and then the bolls were sprayed with a suspension of spores of *Colletotrichum*. Unpunctured bolls were sprayed in a second dish, and bolls which had been punctured with a sterile needle were sprayed in a third dish. After 3 days, infection was observed on all bolls except Sea Island and Cauto. The unpunctured bolls and those which had been pricked with a sterile needle showed an irregular infection. Fungus mycelium and acervuli could be detected on each boll but were not restricted to any particular area. The case was different, however, in the first dish. On all bolls except Sea Island and Cauto, dark areas had formed round the points of inoculation of O. 1 and pink acervuli of *Colletotrichum* could be distinguished in the centre of the dark patch; the whole giving the typical appearance of an anthracnose spot.

It is interesting to note that Nowell(1) reports anthracnose as being secondary to *Bacterium malvacearum* E.F.S. It appears, however, from the foregoing experiments, that O. 1 is capable of preceding *Colletotrichum* but, unlike *B. malvacearum*, rarely parasitises the boll wall extensively as happens in the external boll disease. It is generally the seed that is attacked and it is not until sufficient time has elapsed for autolytic cell separation to take place that other tissue is invaded by the organism; even then the invasion does not extend beyond the infected loculus.

O. 1, therefore, exhibits a weak parasitism but is likely to assume a position of importance in the presence of the anthracnose fungus.

A similar association with *Colletotrichum* was not observed with Y. 3, so that its importance lies in its capacity to produce an internal rot. The organism produces whitish colonies which later become darker and darken most artificial media. It is a rapid grower and appears to be of the same class as that reported from India by Ballard and Norris(3).

The methods of infection and distribution and numerous physiological reactions of both organisms have still to be determined, but it is interesting to note that "stainers" were extremely prevalent in the field from which the original diseased bolls were taken.

DESCRIPTION OF ORGANISMS.

O. 1 (for nomenclature, see Eyre's *Bacteriological Technique* (2)).
Medium. Nutrient agar (Difco + 10° Fuller) + 0·5 per cent. saccharose.
Time. 24 hours.
Dimensions. (Loeffler's methylene blue) 1·8–2·0 × 0·6–0·8 μ.
Flagellum. Single polar.
Gram's stain. Faintly positive or negative.
Gelatine. Not liquefied.

Cultural.

(A) *Plates.* (24 hours.)
Medium. As above.
Colonies. 2 mm. diam., round, umbilicate, refraction weak, hyaline, finely granular, homogeneous, entire, sebaceous, no pigment in medium, glistening, motile.

(72 hours.)
Colonies. 4·5 mm. diam., round or irregular-round, surface contoured, refraction weak, strong in centre, grumose-areolate, entire or undulate, butyrous centre, vitreous margin, glistening, golden-yellow pigment confined to patches in centre.

(B) *Slopes.* (36 hours.)
Medium. As above.
Confluent, regular, flat, alveolate, oleaginous then butyrous, pigment bright yellow restricted to colonies, glistening, motile.
Medium. Potato extract glycerine agar.

Ditto nutrient agar.
Medium. Potato block.
Confluent, regular, flat, surface rugose, homochromous, orange yellow, margin undulate, pale, glistening, pigment not in medium.

(C) *Stabs.* (36 hours.)
Medium. Nutrient agar as above.
Surface as for plates.
Line of puncture. Echinate, growth to bottom of tube. One large gas bubble after 4 days.
Organism is a facultative anaerobe.

(D) *Litmus milk.*
Reaction. Acid then bleached.
Gas. Nil.
Consistency. Coagulated.
Clot. Solid, undissolved in 21 days.
Whey. Abundant, clear.

(E) *Nitrates.* Reduced.

(F) *Indol.* Not produced in 21 days.

(G) *Carbohydrates.*
Dextrose. Reaction acid, gas nil.
Lactose. „ „
Saccharose. „ „
Numerical classification: Bact. 222.22235.

Y. 3.
Medium. Nutrient agar (Difco + 10° Fuller) + 0·5 per cent. saccharose.
Time. 24 hours.
Dimensions. (Loeffler's methylene blue) 0·4–0·5 × 0·3–0·4 μ.
Gram's stain. Positive.
Gelatine. Not liquefied.

CULTURAL.

(A) *Plates.* (18 hours.)
Medium. As above.
Colonies. Round, convex, then amoeboid, effused, refraction strong, amorphous, homogeneous, hyaline, edges entire then lacerate, opalescent then vitreous, glistening, highly motile.

(B) *Slopes.* (18 hours.)
Medium. As above.
Confluent, regular, flat, smooth, refraction weak, amorphous, hyaline, margin undulate-entire, vitreous, glistening, highly motile. Gas in medium.
Medium. Potato extract glycerine agar.
Ditto nutrient agar.
Medium. Potato block.
Confluent and discrete, regular, umbonate, radiating folds, homogeneous, margin erose, cretaceous, no pigment in medium, glistening, motile.

(C) *Stabs.*

Medium. Nutrient agar as above.
Surface as for plates.
Line of puncture. Papillate, medium broken up by gas bubbles, growth to bottom of tube.
Organism is a facultative anaerobe.

(D) *Litmus milk.*

Reaction. Acid then bleached.
Gas. Small quantities.
Consistency. Coagulated.
Clot. Flocculent then solid, undissolved in 21 days.
Whey. Abundant.

(E) *Nitrates.* Reduced.

(F) *Indol.* Not produced.

(G) *Carbohydrates.*

Dextrose. Reaction acid, gas plentiful.
Lactose. ,, ,, gas not so plentiful.
Saccharose. ,, ,, gas plentiful.

Numerical classification: Bact. 222.11110.

Summary.

1. Two organisms have been isolated which are capable of producing an internal rot of at least five varieties of cotton bolls.

2. Inoculation experiments and resulting physiological phenomena are described and the similarity between the two rots demonstrated.

3. The association of one organism with the anthracnose fungus is pointed out and dual infection of a number of varieties of cotton bolls discussed; the resistance of Sea Island and Cauto varieties to the disease is suggested.

4. Detailed descriptions of both organisms and their reactions to various standard media are given.

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(*Received October 30th*, 1925.)

NOTES ON THE CONSTITUTION OF AN UNIMPROVED COTTON CROP

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The greater part of the revenue of the State of Ceará is derived from the sale of the cotton crop and its by-products, and the President of the State, Sr Ildefonso Albano, realising to the full the importance of the cotton crop in relation to the prosperity of the State, started in 1924 a Cotton Service with the object of improving the cotton crop.

Programme of the Work.

On an extensive undeveloped State property about twenty miles inland from the principal town—Fortaleza—a seed farm has been started for the propagation of pure selected seed and for the cultivation of cotton by modern methods under field conditions. Later on it is proposed to have seed farms in three or four different parts of the State for the selection and propagation of seed best suited to that district of which the seed farm would be more or less in the centre. It is proposed that each seed farm should have its own ginning factory, which would gin privately grown cotton as well as that grown on the seed farm.

The lines to be adopted for the improvement of the cotton crop are:

(*a*) Mass selection;
(*b*) Single plant selection;
(*c*) Trial of varieties from other countries;
(*d*) Hybridisation;

but it is on (*a*) and (*b*) that the greater part of the work for several years will be expended.

The present method of cultivation is of the most primitive nature. Except for a little weeding, the soil between the growing plants is left practically untouched. Even the thinning of the plants is omitted, for it is usual to find 8–10 plants struggling for existnce in the same hole, and as many, even, as 18 have been counted. It is owing to this that the crop suffers so much from lack of moisture, for, except during

the early stages, very little rain falls during the growth of the plant. It is very difficult to get accurate figures for the yield per acre, but, even under present conditions of cultivation, it is said to be 190–250 lbs., which compares favourably with that obtained in the U.S.A. With modern methods of cultivation, and with the introduction of pure varieties of seed, the yield per acre should be increased by at least 50 per cent. The seed at present planted is composed of such a mixture of varieties and types that it is extremely doubtful if a similar condition of things exists in any other country. The following botanical varieties are said to be represented: *Gossypium hirsutum*, *peruvianum*, *purpurescens*, *brasiliense*, and *vitifolium*, but it is very difficult to find any plant agreeing in all its characters with those given by Watt. It is no exaggeration to say that every bale of cotton that leaves Ceara contains staple varying from 15 to 40 mm. in length, so that it is obvious that there is room for vast improvement.

An example of how the value of Ceara cotton can be raised by careful picking and ginning is shown by the following report of a well-known firm of Liverpool brokers:

"We find the roller-ginned cotton is rather nice, and if it were possible to get slightly longer staple, ginned in the same manner, we are of the opinion that such cotton would be readily saleable on a much better basis." "Clean cotton, creamy, staple rather cut but of nice character, difficult to value, but might be worth 75 points on."

When the cotton is picked, no trouble is taken to avoid picking dried pieces of leaf, etc., and to keep separate the damaged from the undamaged cotton, and as no steps whatever are taken to clean the cotton before it is ginned, the resulting lint invariably contains foreign matter, with the result that by far the greater portion of the Ceara cotton crop is not worth pass price. The cotton referred to in the above report was part of the ordinary "Herbaceo" crop, the lint not in any way selected, but the crop was carefully picked and any pieces of leaf, etc., that had got into the cotton were picked out before ginning, as is done in Egypt. The gin used was one of Platt's 12-inch roller gins worked by power.

Work done during 1924 and 1925.

In April 1924 some of the best local seed of the variety known as "Herbaceo" was sown on about five acres of land, from which the dense bush had to be cut down and burnt and all stumps grubbed up. Similar areas with one or two other local varieties were also sown, but as their germination was not satisfactory, the bulk of the selection was done on

the Herbaceo crop and all figures given here refer to that crop. Two hundred and twenty-five plants were selected and marked in the field and notes taken on the following characters:

(1) Colour of petals.
(2) Density of the spot, if present, at the base of the petals.
(3) Colour of the pollen.
(4) Length of the style.
(5) Characters of the leaf, stem, and bolls.
(6) Number of vegetative branches.
(7) Number of bolls.

The following figures give the results of the notes taken on the floral characters:

Colour characters of the flowers.

122	plants or	58	per cent.	had white flowers
43	„	21	„	lemon coloured flowers
26	„	12	„	light yellow flowers
18	„	9	„	yellow flowers

The flowers on 16 plants were undetermined.

On the 122 plants with white flowers the characters of the spot on the petal, colour of pollen and length of style were as follows:

Spot on Petal.

114	plants or	93·4	per cent.	had no spot
4	„	3·3	„	a light coloured spot
3	„	2·5	„	a medium „ „
1	„	0·8	„	a deep „ „

Colour of Pollen.

90	plants or	73·8	per cent.	had cream coloured pollen
29	„	23·7	„	yellow „ „
3	„	2·5	„	orange „ „

Length of Style.

1	plant or	0·8	per cent.	had an invisible style
95	„	77·9	„	a short style
23	„	18·8	„	a medium style
3	„	2·5	„	a long style

The above characters were combined in 15 different ways, the 3 combinations containing the greatest number of plants being as follows:

69	plants or	56·6	per cent.	had no spot, cream pollen and a short style
20	„	16·5	„	„ yellow „ „ „
15	„	12·4	„	„ cream pollen and a medium style

On the 43 plants with lemon coloured flowers the characters were as follows:

Spot on Petal.

27 plants or 62·8 per cent. had no spot
9 „ 20·9 „ a light coloured spot
4 „ 9·3 „ a medium „ „
3 „ 7·0 „ a deep „ „

Colour of Pollen.

18 plants or 41·8 per cent. had a cream coloured pollen
19 „ 44·2 „ a yellow „ „
6 „ 14·0 „ an orange „ „

Length of Style.

19 plants or 44·2 per cent. had a short style
21 „ 48·8 „ a medium style
3 „ 7·0 „ a long „

The above characters were combined in 16 different ways, the 3 combinations containing the greatest number of plants being as follows:

10 plants or 23·0 per cent. had no spot, cream pollen and a short style
6 „ 14·0 „ „ yellow „ „ „
6 „ 14·0 „ „ yellow pollen and a medium style

On the 26 plants with light yellow flowers the characters were combined as follows:

Spot on Petal.

8 plants or 30·9 per cent. had no spot
11 „ 42·2 „ a light coloured spot
6 „ 23·1 „ a medium „ „
1 plant or 3·8 „ a deep „ „

Colour of Pollen.

4 plants or 15·5 per cent. had a cream coloured pollen
13 „ 50·0 „ a yellow „ „
9 „ 34·5 „ an orange „ „

Length of Style.

2 plants or 7·8 per cent. had a short style
11 „ 42·2 „ a medium style
13 „ 50·0 „ a long „

The above characters were combined in 15 different ways, the 4 combinations containing the greatest number of plants being as follows:

4 plants or 15·4 per cent. had a light coloured spot, yellow pollen, and a long style
3 „ 11·6 „ no spot, yellow pollen, and a medium style
3 „ 11·6 „ a light coloured spot, orange pollen, and a long style
3 „ 11·6 „ medium coloured spot, yellow pollen, and a long style

On the 18 plants with yellow flowers the characters were combined as follows:

Spot on Petal.

1 plant or	5·6	per cent.	had no spot
4 plants or	22·2	,,	a light coloured spot
4 ,,	22·2	,,	a medium ,, ,,
9 ,,	50·0	,,	a deep ,, ,,

Colour of Pollen.

8 plants or	44·4	per cent.	had yellow pollen
10 ,,	55·6	,,	orange coloured pollen

Length of Style.

1 plant or	5·6	per cent.	had an invisible style
3 plants or	16·7	,,	a medium ,,
14 ,,	77·7	,,	a long ,,

The above characters were combined in 8 different ways, the 2 combinations containing the greatest number of plants being as follows:

4 plants or	22·1	per cent.	had a	deep	coloured	spot,	orange	pollen,	and a	long	style
3 ,,	16·7	,,	,,	light	,,	,,	,,	,,	,,	,,	,,

It will thus be seen that there were 55 different kinds of flowers amongst 209 plants and it will also be noticed that:

(1) Flowers with white petals have, generally, no spot at the base of the petals, cream coloured pollen, and a short style.

(2) Flowers with lemon coloured petals have, generally, no spot, cream or yellow coloured pollen, and a short or medium length style.

(3) Flowers with light yellow petals have, generally, a light coloured spot, orange or yellow coloured pollen, and a long or medium style.

(4) Flowers with yellow petals have, generally, a deep coloured spot, orange coloured pollen, and a long style.

Leaves.

They varied from glabrous to densely pubescent and from having 5 long and narrow lobes to 2 short and broad lobes.

Stem.

It varied from glabrous to densely pubescent and from the true monopodial type to the sympodial type with no monopodial branches.

Bolls.

They varied in shape from round to long and pointed, in colour from light green to dark green, in locks from 3 to 5, and in number from 5 to 124.

After the cotton had been picked each of the 225 samples was examined for:

(1) Total weight of seed cotton.
(2) Percentage of damaged cotton.
(3) Length of the fibre.
(4) Colour of the fibre.
(5) Strength of the fibre.
(6) Fineness of the fibre.
(7) Ginning out-turn.
(8) Seed characters.

Weight of Seed Cotton.

It varied from 2 to 448 gm.

Percentage of Damaged Cotton.

It varied from 0 to 66·6 per cent., being lowest for the plants that opened their bolls quickly and highest for those plants that continued opening their bolls for many weeks.

Length of Fibre.

2	plants or	0·9	per cent.	had	fibre	18–19	mm.	in length
5	,,	2·2	,,	,,	,,	20–21	,,	,,
44	,,	19·6	,,	,,	,,	22–23	,,	,,
93	,,	41·4	,,	,,	,,	24–25	,,	,,
45	,,	20·0	,,	,,	,,	26–27	,,	,,
27	,,	12·0	,,	,,	,,	28–29	,,	,,
6	,,	2·6	,,	,,	,,	30–31	,,	,,
1	plant or	0·4	,,	,,	,,	32–33	,,	,,
2	plants or	0·9	,,	,,	,,	34–35	,,	,,

Colour of Fibre.

137	plants or	60·9	per cent.	had	a light cream fibre
58	,,	25·8	,,	,,	white fibre
27	,,	12·0	,,	,,	cream fibre
2	,,	0·9	,,	,,	dark cream fibre
1	plant or	0·4	,,	,,	brown fibre

Strength of Fibre.

138	plants or	61·3	per cent.	had fibre medium in strength
66	,,	29·3	,,	weak fibre
21	,,	9·4	,,	strong fibre

Fineness of Fibre.

The fibre of	182	plants or	80·9	per cent.	was	medium
,, ,,	25	,,	11·1	,,		fine
,, ,,	18	,,	8·0	,,		rough

Ginning Out-turn.

3	plants or	1·4	per cent.	had a ginning out-turn from			16–20	per cent.
7	,,	3·1	,,	,,	,,	,,	21–25	,,
98	,,	43·5	,,	,,	,,	,,	26–30	,,
110	,,	48·9	,,	,,	,,	,,	31–35	,,
7	,,	3·1	,,	,,	,,	,,	36–40	,,

Seed Characters.

There were over 20 distinct varieties of seed varying from seeds thickly covered with fuzz to seeds with no fuzz. The fuzz varied in colour from white to brown and green.

It is interesting to note that by correlating the length and strength of the fibre with the colour of the flower that the best fibre is produced on plants with the darkest coloured flowers, as the following table shows:

Length of Fibre.

	White flowers	Lemon or yellow flowers
Plants having fibre 18–19 mm.	100 per cent.	—
,, ,, ,, 20–21 ,,	100 ,,	—
,, ,, ,, 22–23 ,,	89 ,,	11 per cent.
,, ,, ,, 24–25 ,,	60 ,,	40 ,,
,, ,, ,, 26–27 ,,	44 ,,	56 ,,
,, ,, ,, 28–29 ,,	12 ,,	88 ,,
,, ,, ,, 30–31 ,,	9 ,,	91 ,,
,, ,, ,, 32–33 ,,	—	100 ,,
,, ,, ,, 34–35 ,,	—	100 ,,

Strength of Fibre.

	White flowers	Lemon or yellow flowers
Plants with weak fibre	63 per cent.	37 per cent.
,, ,, medium fibre	55 ,,	45 ,,
,, ,, strong fibre	23 ,,	77 ,,

In addition to the 225 plants already referred to, a further 350 single plant samples were also examined for the same characters, and from the total number of plants, 254 were selected for sowing in 1925, differing in the length of staple, ginning out-turn, time of maturity and other characteristics. Every one of the resulting plants (the total number being about 20,000) was examined for the various field characters, and in the majority of cases the lint was roughly examined before picking, so that any variety giving inferior results was immediately discarded. As another instance of the extraordinary mixture of cottons in the State, I may mention that not more than 12 of the originally selected plants were found breeding true to type, the progeny of some of the selections containing a dozen or more different types, differing in the field, lint and seed characters from the parent plant.

In addition to the foregoing single plant selection work, a certain portion of the crop was set aside for mass selection, that is, all plants with field characters obviously different from the bulk of the "Herbaceo" plants were pulled up and destroyed; then, after the cotton was ginned, only those seeds were kept which were completely covered with a grey or white fuzz, as being the true "Herbaceo" type of seed.

Though the work has not yet been in operation for two years, the results so far obtained are most satisfactory, and show that Ceara is capable of producing cotton equal in value to that of Egypt, if full use is made of the potentialities of the country.

Summary.

This paper gives an account of the formation of the Cotton Service in Ceara, one of the north-east states of Brazil, the lines on which the work of improving the cotton crop is to be conducted and some figures showing the composition of the crop prior to any selection.

Owing to the large number of types represented and the long period of time during which cross-fertilisation has taken place, several years must necessarily elapse before any pure selected strains can be put on the market.

(*Received February 2nd*, 1926.)

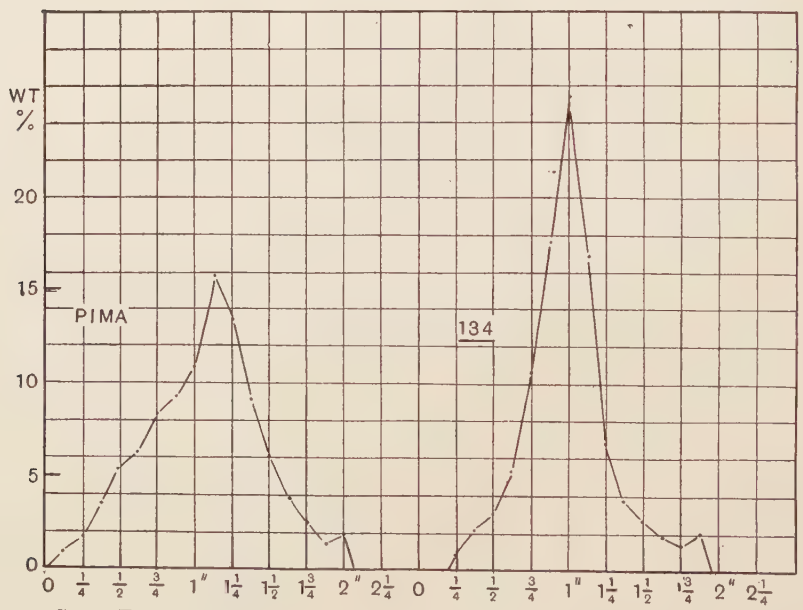
APPENDIX

REPORT OF HAIR TESTS ON BRAZILIAN SAMPLES, 1925

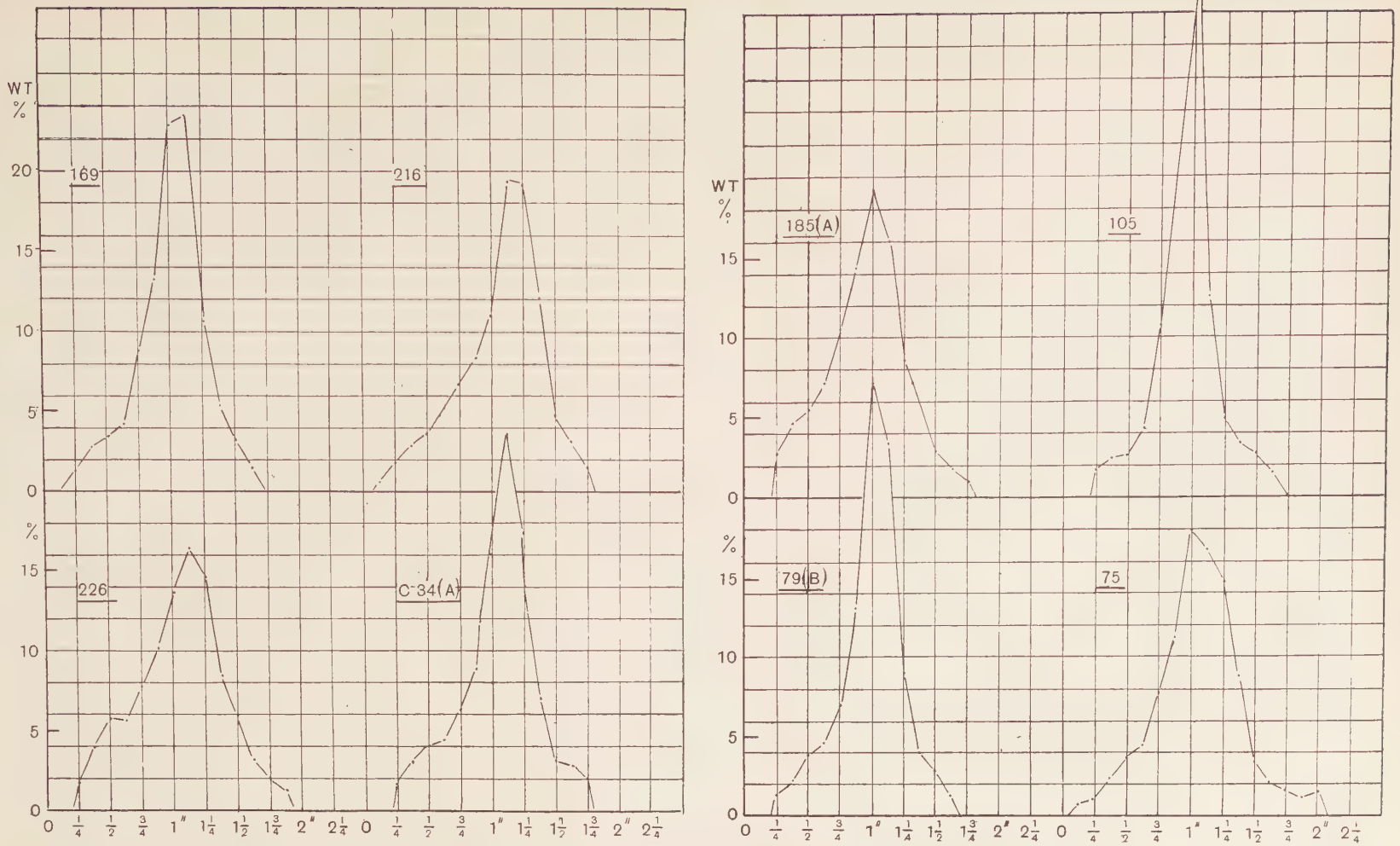
(With 3 Text-figures.)

The samples were tested for hair length, strength, weight, ribbon width and wall thickness. Attached are diagrams showing the composition of each sample with regard to hair length; the other measurements are given below.

Sample	Weight (mg. per cm.)	Strength (gms.)	Strength/ weight ratio	Ribbon width (mm.)	Wall thickness (mm.)
79 B	·00889	5·3	2·95	·0232	·0065
105	179	4·5	2·52	246	73
216	189	5·9	3·12	246	73
134	216	5·7	2·74	247	70
185 A	166	4·6	2·77	235	65
75	180	4·7	2·61	219	65
169	180	5·4	3·00	208	62
C 34 A	168	5·0	2·98	236	65
226	191	5·7	2·98	238	73
Pima	102	4·2	4·12	198	57



Sorter Tests on Brazilian Samples (1925) per B. G. C. Bolland, Dec. 1925.



Sorter Tests on Brazilian Samples (1925) per B. G. C. Bolland, Dec. 1925.

Hair length. All the samples lie between 1″ and $1\frac{1}{4}$″; nos. 169, C 34 A, 105, 79 B and 134, are exceptionally regular, 226 and Pima have rather wide variation for their model length, but the other curves resemble American staple types.

Weight per cm. This figure may be used as an indication of fineness but is not a measure of it. Pima is exceptional, being as fine as the finest Sea Island; 134 is rather coarse, and the other sample is about the same as American.

Strength weight ratio is proportional to the hair strength corrected for weight per cm. and indicates hair strength. For this ratio 4·0 is a high value, as in the case of Pima, 3·0 is good, 2·5 is low.

Ribbon width and wall thickness combined give some idea of the cross-sectional area of the hair. All the samples are rather coarse; Pima is not so fine as the hair weight figure would lead one to expect, but is finer than most of the others. Its low hair weight thus appears to be due to density, which may be a result of agricultural factors.

E. Lanigan,
Laboratory Section,
(*Testing Room*).

Fine Cotton Spinners' and Doublers' Association, Ltd.,
Experimental Department,
Bollington, Cheshire.

(*Received from Mr B. G. C. Bolland, December,* 1925.)

CONCERNING "FAIRY RINGS" IN PASTURES

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(With 1 Text-figure.)

In the year 1911 I published the results of an investigation of two fairy-ring-forming fungi(1) which are commonly met with in pastures—*Marasmius oreades* (Fr.), the fairy-ring champignon, and *Clytocybe gigantea* (Sow.). I have since observed other fairy rings, caused by fleshy fungi, in order to compare their growth with those already studied, which have ceased to exist.

In 1922 Professor Buller called my attention to the work of Shanz and Piemeisel on the "Fungus Fairy Rings of Eastern Colorado" and their effect on vegetation(5). These authors, in addition to their own investigations, give an exhaustive epitome of the work that had hitherto been published on fairy-ring fungi, referring to notes or investigations by 31 authors in connection with 47 fleshy fungi. Since the observations and conclusions of these authors, especially with regard to the cause of the death of the vegetation, do not always agree with mine and those of other investigators, a short review and criticism of the subject of "fairy rings" may not be without interest.

Shanz and Piemeisel distinguish three types of fairy rings in Eastern Colorado—I have noted the same three types in England.

They are:

Type I, those in which the grass is killed or badly damaged;
Type II, those in which the grass is only stimulated;
Type III, those which produce no effect upon the grass.

Type I. This is perhaps the most interesting and the one which has been investigated most frequently, as it appears on lawns, bowling greens and golf courses, or in pastures. *Marasmius oreades* (Fr.) is a good example in England of this kind, but according to Shanz and Piemeisel not in America. Such rings may often be met with in poor pastures, where about June the full effect of the presence of the fungus on grass may be seen.

Three zones (Fig. 1) may be noted to such rings:

(*a*) an inner zone where the grass is deeper green and much more luxuriant than the grass within the ring, and that of the pasture generally,

(*b*) surrounding this, a zone of dying or dead grass, and

(*c*) a narrow outer zone of stimulated grass situated externally to (*b*). This outer zone gradually broadens until about towards the end of September it may be even broader than zone (*a*).

A sod of the middle zone is well permeated with fungus hyphae to the depth of about one foot, visible even to the naked eye, and dense wefts of fine white hyphae cling round dying roots, and when atmospheric conditions are very humid mass on the surface of the soil. Under high magnification the fungus mycelium can be seen penetrating the roots of the grass, the soft parenchymatous cortical parts of which it entirely consumes.

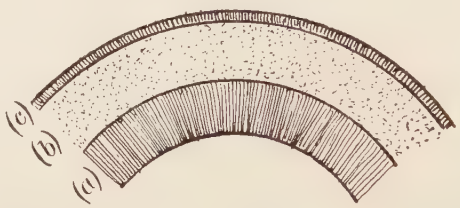

Fig. 1. Diagram showing the different zones of a fairy ring, caused by *M. oreades*. (*a*) Inner dark green grass zone. (*b*) Dead grass zone. (*c*) Outer dark green grass zone.

A curious physical phenomenon is also to be noted in this zone, that is, the resistance offered to the percolation of water; hence the soil of this zone is always dry compared with that of the zones on either side or in the field generally. This I have proved elsewhere (1) to be due to the air entangled within the meshes of the mycelial network occupying the spaces between the particles of soil. On this zone the sporophores of the fungi appear, usually on the inner side of it, but frequently in other parts, about a month after the dying down of the grass.

In a sod of zone (*a*)—the innermost zone—the mycelium is present in far less quantity than in the middle zone, being scarcely visible to the naked eye and in an impoverished and dying condition. The luxuriance of the grass growing on this zone appears to be due to the fertilising qualities of the decomposition products produced partly by the fungus in destroying the plants (grass and others) of this area and partly as the result of the decomposition of the mycelium itself.

The rich dark green appearance of the grass on the outer narrow

zone (*c*) is due to the stimulating action of the fungus when it begins its attack on the grass, for as with many other parasites stimulation of growth is often the first effect of the parasite on the host. The amount of mycelium on this zone at first is not great and although rootlets are continually being destroyed there is evidence (1) that more are rapidly formed. No doubt the grass is further stimulated by the saprophytic action of the fungus rendering available the products of decomposition of any dead rootlets occurring among the living plants of the sod.

During late autumn and winter these fairy rings are scarcely visible, for the dead grass zone recovers its vegetation: owing to the penetration of neighbouring rhizomes and to the germination of seeds.

Hence in a fairy ring of *type I* we see a regular annual progress of a parasitic fungus living on grass in the soil and gradually proceeding outwards, fairly uniformly in all directions from the centre, where it started, presumably from the germination of a spore. As it advances it destroys the roots of the grass and finally after killing the plants consumes all the softer tissues. The mycelium now being without food and having rendered this area unsuitable for further growth by the secretion of toxic (staling) substances, produces sporophores and then dies. The soil of this zone is thus much enriched and when a new growth of grass appears, it shows a luxuriant growth. Meanwhile hyphae continue to grow forward, attack a new zone and the same sequence of events is repeated. The sequence extends over three years; in the first year the grass is being attacked, in the second it succumbs to the attack, in the third year a new crop of grass benefits from decomposition products rendered available by the mycelium which killed the preceding crop of grass and also from the decay of the mycelium itself.

Clytocybe gigantea (Sow.) is another fungus which must be included in *type I*: these rings usually appear three or four weeks later than those of *M. oreades*; they do not fruit every year, nor is their fruiting season so prolonged as the former. The dead grass zone does not recover its vegetation so quickly; one that I observed for three years (1) showed the bare zone for a whole year instead of two or three months—the period usual in the case of *M. oreades* rings.

It seems highly probable too that the rings of *Tricholoma gambosum* (St George's mushroom) belong to *type I*, since a zone of dead grass usually appears in connection with these rings.

Shanz and Piemeisel have paid most attention to the rings formed by *Agaricus tabularis* (Peck) (a fungus allied to *A. arvensis*) which also belongs to *type I*. The only point of difference between these American

rings of *A. tabularis* and the English *M. oreades* is in the position of the sporophores which are said to come up on the outer stimulated zone instead of as in the case of the English *M. oreades* anywhere on the bare zone.

A very curious feature to be noted in connection with the fairy rings of Eastern Colorado is that *M. oreades* does not behave like *type I* there: it does not do any damage to the grass and therefore in America must be classed as *type II*. All investigators of *type I* agree that these rings are associated with poor pastures and discouraged by high nitrogenous manuring.

Much discussion has arisen as to the cause of the outward progression. Why does the fungus progress as a ring not as a disc? Why does it never grow backward and attack the grass again in the centre of the ring?

In my researches of 1909–11 I have shown that the fungus excretes something toxic to itself (staling substances(1)). The presence of this together with the exhaustion of the nutrient material in the soil would account for the fungus growing outward into a more favourable zone, and would also account for the dying out at the point of intersection of two meeting rings. The fact that these rings are usually found in poor pastures also has some bearing on this point. I have proved in the case of both *M. oreades* and *Clytocybe gigantea* that if the grass in front of an advancing ring is manured with ordinary stable manure the rings die out; if manured only in patches the rings die out in patches. Doubtless in poor pastures the grass being of feeble growth falls an easy victim to the parasitic fungus, but when stimulated to active growth by nitrogenous manure it resists the onslaught of the parasite. The fungus never grows backward into the zone it has left because it would first have to encounter certain staling substances, which bar the way, and even if these were not there, the now very vigorous plants occupying this zone are not likely to yield easily to the attack of a parasite.

Most investigators have drawn attention to the fact that the soil of the middle zone of a fairy ring, especially during late spring and summer, always contains relatively less moisture than the zones on either side and also than the soil of the field generally, and several, including Ritzen Bros.(5) (1901) and Molliard(4) (1910), attribute the death of the vegetation to this dry condition of the soil. Shanz and Piemeisel also consider this the cause of the death of the vegetation noted in the rings formed by *A. tabularis*—the fungus hyphae prevent the percolation of rain water and the intense drought produced leads to the death of the vegetation: these authors consider that no harmful effect could be noted

in these rings so long as the soil contained available water. This is quite contrary to my observations in the case of *M. oreades* rings. As I have already stated, I attribute the death of the vegetation to parasitism on the part of the fungus, a conclusion to which Ballion has also arrived.

In my researches of 1909–11 various experiments (1) pointed to the presence of a toxic excretion produced by the fungus which damaged the roots of the grass and enabled the fungus to attack them even before they were dead. Since reading the work of Shanz and Piemeisel (5) I have repeated my investigation of the soil in connection with the fairy rings caused by *M. oreades* and I confirm my statement that among the roots of the grass plants on the outer edge of the outer dark green zone the first attack of the fungus mycelium on the rootlets can be seen and that it is indicated by a brownish discoloration of the delicate root tips or of the external cortical tissue near this region, and by loss of symmetry of the root hairs. Very little mycelium is present and as this increases discoloration becomes more pronounced, and I would emphasise that this is at a time when the moisture content of the soil is practically normal, for it is seen on the advancing edge of the ring. If plants just behind this area be examined during January and February when the moisture content of a fairy ring is much the same as that of the field generally, and compared with plants taken from normal turf, no difference will be apparent except at the roots when it will be seen that these when washed free from soil, look rusty coloured because of the numerous dead and dying rootlets.

There is evidence that the fungus is producing a toxic effect upon the young rootlets. In experiments where uninfected roots have been left 24 hours in very damp infected soil, the rootlets turn brown or black: in a control experiment using uninfected soil, the root tips remained unchanged and uninjured: the toxin seems to be injured by heat, since the effect is far less pronounced when the soil is sterilised first. Young roots also turn black when allowed to remain 24 hours in an extract of infected soil from which all soil and fungus mycelium have been filtered off. In the controls of boiled extracts or of extract from uninfected soil the roots remained uninjured. Infected roots, besides having the rusty appearance, always show more copious branching which points to an effort on the part of plants to make up for the destruction of root apices which is constantly going on.

Since in this outer infected zone, during autumn, winter and spring, the mycelium is becoming more abundant, a stage is arrived at, usually about June, when the plants can no longer react (by forming more roots)

against the attack of the fungus, and then a zone of dead grass appears. Doubtless drought may assist in hastening on this stage, but I do not think it is the primary cause. Further, I have seen this advancing zone of infected grass dying down rapidly when the soil has been quite moist, and had been so for weeks previously.

The observation that fairy rings thrive best in dry seasons seems quite in accord with what might be expected, for mycelium in soil would bring about such a condition that the little rain that falls would not soak in and thus the vitality of the grass would be lowered and consequently conditions would be more favourable for the parasitic fungus. It seems quite possible that in dry seasons this zone might be so lacking in moisture that some plants would really die owing to drought.

I have mentioned(1) that in some years it is quite possible for the mycelium to grow outwards and only occasionally be present in the soil in sufficient quantity to kill the grass; I have seen cases where part of a ring may not die down at all one year and yet the ring as a whole may advance fairly uniformly. This has happened in an unusually damp year when the growth of the grass has been favoured by the moist weather conditions, as well as by the presence of certain decomposition products rendered available by the activity of the fungus mycelium present, and although some of the roots have succumbed to the toxic effect of the fungus, in this case the host has kept the upper hand, formed more roots and flourished.

Wet seasons must also act to the advantage of the grass and against the fungus because the toxins excreted by the fungus, being soluble, are continually draining away. In agreement with this but yet to a certain extent supporting the view that drought is the chief factor in bringing about the death of the grass is that, in the neighbourhood of Birmingham, fairy rings are far more common and show better development on sandy soils than on clay soils; but this observation also supports my view, for grass being more vigorous on clay soils, owing to clay being more retentive of moisture, is not likely to suffer so much from attacks of *M. oreades*.

Shanz and Piemeisel unfortunately do not give any details of microscopic examinations of *A. tabularis* rings beyond the abundance or otherwise of mycelium in the different zones of the ring. It would be interesting to know if any difference is distinguishable between the roots of grass in normal and in infected soil such as I have noted in connection with *M. oreades* rings.

That the effect of *M. oreades* on grass in Eastern Colorado should be so different from its effect in England and elsewhere is a matter of

surprise and interest. According to Shanz and Piemeisel, although it forms fairy rings, the only effect produced on the vegetation is to stimulate growth, a zone thus being produced, which may be compared to the outer stimulated zone of the ring of *A. tabularis*.

These authors report that only a small amount of mycelium is developed and that it is comparatively difficult to distinguish the threads in the soil; this is at variance with observations in England where a bare zone is formed and the mycelium is so abundant in the soil underneath this that it can be seen with the naked eye, and often before the dying down of the grass can be plucked like fragments of cotton wool from the surface of the soil.

Also, the production of the sporophores on the advancing edge of the stimulated zone, where the mycelium must be least abundant seems an unusual procedure, and different from the course of events in England, fruit bodies being produced where mycelium is most abundant.

In this stimulated area authors consider the fungus mycelium is reducing and living on the products of the organic matter in the soil: this may take place to a certain extent, but the parasitic action of the fungus destroying and decomposing the rootlets also accounts for some of the stimulation. If the stimulation were chiefly due to the reduction of the organic matter in the soil, surely when the advancing edge of a ring were treated with organic manure on which it is possible for the fungus to live saprophytically instead of the ring being destroyed the fungus would benefit. On the contrary, rings thus treated have disappeared, and when the rings were only treated with manure in patches, only the patches disappeared: grass flourished to the detriment of the fungus.

That the fungus can live as a pure saprophyte I have proved by growing the mycelium on various artificial media and on sterilised green grass.

Type II. The ring of *type II*, in which the grass is only stimulated, no damage apparently being done, is well illustrated in England by the *Tricholoma personatum* (Fr.), *T. nudum* (Bull.) and *T. panaeolum* (Fr.) rings.

Usually the sporophores of these rings appear during the autumn, but in 1923, on Ladbrooke Park (Warwickshire) golf course, a very perfect ring caused by *T. personatum* produced countless sporophores during January 10th to 23rd (the season was a mild one). In this case the presence of the fungus had been somewhat detrimental to the growth of the grass, for the grass of the fairway, after being cut in August 1922,

had grown well except on the actual ring (width 1½ to 2 ft., diameter of ring about 5 yards), where though very short it was conspicuous as regards its dark green colour. An examination of the soil under these *Tricholoma* rings reveals only a very small amount of mycelium, which does not cling about the roots of the living grass as the mycelium of the *M. oreades* does.

The fungus here is evidently not behaving as a parasite but living saprophytically on the humus and dead rootlets in the soil as it progresses: the stimulation generally seen being doubtless due to the products of decomposition rendered available to the grass by the fungus.

A pasture in which large rings of *T. panaeolum* appeared during the autumn of 1910 and of 1911 was heavily manured and a crop of grass taken off the following June. This treatment did not favour the growth of the fairy rings, for they never appeared again: the high nitrogenous manuring of the land by encouraging a vigorous growth of grass acted detrimentally to the further growth of the fungus.

T. personatum and *T. nudum* are met with in woods as well as in pastures growing saprophytically on dead leaves where they sometimes form fairy rings. Being edible, they are cultivated in France and elsewhere on beds of oak and other leaves, the acidity of such a bed being to a certain extent neutralised by adding a little stable manure.

Rings of *Lycoperdon gemmatum* (Batsch.) and *Agaricus arvensis* (Schoeff.) also belong to *type II*, and *Agaricus campestris* (Linn.) both in England and in Eastern Colorado conforms to this type.

Type III. Among rings of *type III*—those which do not affect grass—may be counted *Hebeloma crustuliniforma* var. *minor* (Bull.) and *Tricholoma sordidum* (Fr.). A very perfect ring of the former, 38 in. diameter, appeared on my lawn in August 1922 but never reappeared. The ring did not affect the grass at all. Abundance of mycelium was visible just around the base of the sporophores mixed up with dead rootlets and moss, but the ordinary sod underneath the ring did not show more mycelium than is usual everywhere in turf, in fact was quite normal.

Two rings of *T. sordidum* appeared on the same lawn in 1921 and lasted three years. An examination of the turf showed that the fungus was living saprophytically at and very near the surface of the ground. There were indications that the fungus lived on dead moss and dead root hairs of grass.

These rings, which were only about 22 in. inner diameter when first noticed and from 6 to 12 in. wide in the ring area, progressed from 12 to

18 in. during the second year. During the third year, instead of having the grass cut, it was allowed to grow in order to observe the effect on the fungus; only a few sporophores appeared and in 1924 there was no trace of the ring; it may have been that grass living normally under good cultural conditions instead of being continually cropped excreted something toxic to the fungus.

Chemical Changes produced in Soil.

Concerning the chemical changes produced in soil during the progress of fairy-ring fungi very little work has been done.

Lawes, Gilbert and Warrington (1883)(2), and Molliard (1910)(4), investigated some of the chemical changes occurring in connection with *M. oreades* rings, while Shanz and Piemeisel (1917) give some details of a chemical investigation of the rings formed by *A. tabularis* (Pk.) and *C. cyathiformis* (Bosc.). Lawes, Gilbert and Warrington investigated fairy rings in order to determine the source of the nitrogen of the fungi, which apparently were the cause of a natural rotation of crops. They found that there was more nitrogen outside the ring, in the ordinary sod, than on the ring or inside, but that nitrates were far more abundant on the ring and they concluded that the source of the nitrates was the organic matter of the soil.

Molliard(4) concluded that the mycelium acted directly on the organic matter of the soil, giving rise to ammoniacal salts; he found most ammonia under the dead grass zone and noted very little difference between the amount present outside and inside the ring.

As regards the ammonia content Shanz and Piemeisel agree with Molliard that most is to be found in the ring area, but less without than within the ring. With regard to nitrates the figures of Shanz and Piemeisel seem rather too variable to draw conclusions from, but these authors summarise by saying that ammonia is more abundant in the soil well permeated with mycelium, whereas nitrates are more prevalent in the part occupied by the old dying mycelium.

Now when determining the *total* nitrogen (in any form) both Lawes, Gilbert and Warrington, and Shanz and Piemeisel, found more outside the ring in the ordinary soil than on it, and concluded that the source of the nitrogen bringing about the stimulation of growth of fairy rings is the organic matter of the soil, which, owing to the activities of the fungus mycelium, gives up its nitrogen in a form such as ammonia and nitrates which can be easily assimilated by grass, hence that nitrogen is only changed from one form to another.

Through the kindness of Professor Ling and Mr A. Brookes of the Biochemical Department of the Birmingham University, I obtained the following determination of the nitrate and ammonia content of samples of soil taken from three different zones of a fairy ring (*M. oreades*)[1] and from the normal soil outside it.

	Total nitrogenous matter %	Ammonia salts and free ammonia %	Nitrates and nitrites	Moisture %
Inner stimulated zone (*a*)	0·243	0·00375	Nil	16·25
Dead zone (*b*)	0·253	0·00326	,,	18·84
Outer stimulated zone (*c*)	0·204	0·00196	,,	20·10
General field	0·218	0·00117	,,	20·76

The soil samples were taken by boring to a depth of about 12 in. with a sharpened gas pipe (inner diameter 1 in.). Three borings were taken from each zone and from the surrounding field. The soil from the ring had a particularly offensive smell, especially in the dead zone. The total nitrogenous matter (not including nitrates and nitrites) was estimated by the method of Ling and Price (3). The ammonia salts and free NH_3 were determined by shaking the soil with distilled water and distilling the extract with 40 per cent. soda and nesslerising as in the micro-kjeldahl method. In determining the nitrates and nitrites the extract used for the ammonia salts and free ammonia was employed. The salts were reduced by means of aluminium amalgam, distilled with 40 per cent. soda and nesslerised as before: the results give the total nitrogen in the following forms: ammonia salts, free ammonia, nitrates and nitrites. In order to determine the nitrates and nitrites the percentage of ammonium salts and free ammonia is deducted from the percentage of total nitrogen.

The results in columns 4 and 5, *i.e.* the finding of no oxygenated derivatives of nitrogen, and the high moisture content, seem rather unusual, but the samples were taken in August 1922, after there had been heavy rains for several weeks and the ground was thoroughly soaked, so that any nitrates probably drained away in solution as quickly as formed. Samples taken in July from the same ring gave the usual results as regards moisture:

Inner stimulated zone	8·82	per cent.	water
Dead zone	5·74	,,	,,
Outer stimulated zone	5·79	,,	,,
General field... ...	12·76	,,	,,

[1] Only a small portion of this ring and very few sporophores appeared in 1923, and no trace of it was seen in either 1924 or 1925.

It will be noted that the ammonia content is highest in the area of the inner stimulated zone and is very nearly the same under the dead zone; judging from the figures given by Molliard, and Shanz and Piemeisel, the ammonia content is usually highest under the dead zone, but the tables of Shanz and Piemeisel show instances parallel with the above: perhaps this may be correlated with the finding of no nitrates in the inner stimulated zone, which is usually the zone associated with an abundance of nitrates, bacterial action there usually changing the ammonia salts formed in the previous year into nitrates. But it is rather remarkable that the outer stimulated zone should have less nitrogen taken as a whole than is to be found in the field generally: perhaps this may be due to the fact that the outer stimulated area is producing a good crop of grass and so uses more nitrogen compared with that used in producing the normal poor crop of the field.

It will also be noted that the dead zone and inner stimulated zone have gained nitrogen, since the total amount of nitrogen outside the ring is less than in these zones; this does not agree with Lawes, Gilbert and Warrington's results or with most of the results of Shanz and Piemeisel.

Although the results of Lawes, Gilbert and Warrington and of Shanz and Piemeisel point to the conclusion that there is no fixation of nitrogen by fairy-ring fungi, but only a change of the nitrogen into a more easily assimilable form, it seems desirable that culture experiments should be made in order to settle this point. The mycelium of *M. oreades* and other fairy-ring fungi can easily be grown on artificial media so that the problem should not be a difficult one for a chemist.

In conclusion I would add that I consider a fairy-ring fungus such as *Marasmius oreades* (in England) behaves as a parasite and in its progress destroys grass by some toxic excretion, and the fungus lives saprophytically on the dead organic matter thus produced and on any other already present in the soil. The grass which appears on this dead zone the year following the destruction, owes its luxuriance to the easily assimilable nitrogenous compounds resulting from the activities of the fungus mycelium, in its destruction of the grass, and also to similar compounds produced by the decay of the mycelium itself. Further, during several months of the previous year this zone did not support a crop, and the soil here, in dry years especially, does not suffer the usual loss from the draining away of soluble food substance.

SUMMARY.

1. Observations are made on the growth of three types of fairy rings found in pastures and elsewhere.

2. *Marasmius oreades* belongs to a type which causes the death of rings of grass. It behaves as a parasite, destroying grass by means of a toxin.

3. Comments are made on the views of Shanz and Piemeisel, who consider the death of the grass in certain fairy rings is merely due to a drought produced by the presence of mycelium in soil.

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(*Received September 28th*, 1925.)

NOTE ON THE OCCURRENCE OF DISEASED SHOOTS ARISING FROM POTATO-TUBERS INFECTED BY *PHYTOPHTHORA INFESTANS*

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(With Plate V.)

For more than 60 years the problem of the perpetuation of the potato-blight fungus (*Phytophthora infestans*) from year to year has exercised the minds of mycologists. By analogy with the life-history of closely related fungi, two methods of hibernation have been considered possible: (1) the fungus may exist in the form of oospores which on germination the following year attack the potato plant and cause the first outbreak of disease for that season; or (2) the fungus may hibernate in the form of mycelium within the potato tuber and grow out into a new shoot and there produce spores. So far, however, oospores have never been found in the living or dead tissues of the potato plant, and their production has only been observed when the fungus has been cultivated artificially on a certain nutrient medium.

Supporters of the theory that the formation of oospores must take place in nature and that this is the normal, common method of the perpetuation of the disease from year to year point out, as justifying their belief, the fact that experiments proving that the disease can arise each season on shoots produced from infected seed tubers have been very few in number—the authentic cases being apparently but four. We have now to record a fifth case—the first observed in this country. Before giving the details of our experiments, we will review briefly and critically, the work, relevant to the present subject, of the previous investigators, viz. de Bary, Jensen, Dr Pethybridge and Prof. Melhus.

De Bary's experiments were of two kinds: (1) in 1861 with naturally infected potato-tubers, grown either in the house or in the laboratory; and (2) in 1875 with artificially infected tubers, planted in a garden.

De Bary's earlier observations(1), which are unaccompanied by any

details of the experiments, are as follows: When a tuber containing the mycelium sprouts, the mycelium grows into the shoots, accompanying them as they lengthen and reach the surface of the ground. If the mycelium has entered the shoot in large quantities then the latter is soon killed; with diseased tubers which have sprouted, shoots one to several inches long are not uncommonly found which have turned black and the parenchyma of which is permeated by abundant mycelium, which, by the use of well-known technique, can be induced to form fructifications. If a less amount of mycelium has entered the shoot then the latter appears externally completely healthy and shows internally only a few cells which have turned brown, although the presence of the fungus in the shoot is very easily proved.

In his later paper (2) de Bary observes: "...tubers containing *Phytophthora*, when they are growing, not unfrequently send out shoots into which the fungus passes from the tuber. The fungus advancing slowly in its growth, at last kills the shoots which, for the most part, were always in a sickly condition. The same tubers, as is known, may also send out healthy shoots. At the same time I further showed, that under special circumstances the fungus in these diseased shoots develops conidia, which become centres for the further spread of the disease. These were not conjectures, but facts observed in experiments."

The single experiment of which details are given is as follows: In March about 50 healthy potatoes were inoculated at the eyes with fresh conidia. On April 2nd these tubers were planted "in common garden-soil, in a box without a bottom, and open to the air," in a garden "in the town, far from the fields." Whilst many of the tubers (including those known to be diseased) produced healthy foliage, one plant "was specially distinguishable from the rest, because the six shoots which it sent above ground remained in a wretched condition." On May 12th these had become brown. One was cut off for microscopic examination and found to contain the mycelium, which was subsequently found to be present also in the parent tuber. The other five shoots produced no conidia until May 18th, when, following a warm night of heavy rain, the stalks and petioles of the five shoots were found to be thickly covered by conidiophores with mature conidia. On May 21st secondary infection on neighbouring healthy plants was first observed; "two leaflets on the upper part of a branch, which was near the five sickly shoots," producing spots with conidia on the lower surface. "From May 25th onwards, the fungus spots were to be seen plentifully scattered without order on the stalks, petioles, and leaves of all the plants. About the same time

several other diseased tubers also gave off small shoots, into which the mycelium of the fungus had passed from the tuber; no further observation, however, was made on them, because the disease was then far advanced everywhere."

J. L. Jensen in 1887 published(3) a lengthy treatise on potato blight and its control, and like de Bary, gave a very clear description of the life-history of the fungus. One of the principal objects of his paper was to set forth his ideas on the subject of potato "seed" disinfection and in support of his claims as to the practical value of the process, he carried out experiments with diseased tubers, both to repeat the work of de Bary and to demonstrate that it was indeed by planting such tubers that the disease was enabled to continue from one year to the next.

The following experiment was carried out in 1883 in a garden at Neuilly. Fifty healthy tubers and 50 diseased tubers were planted at a depth of 10 cm. in two double rows, the healthy being only 10 cm. from the diseased and the double rows 63 cm. apart and the same distance from two similar double rows planted also with 50 healthy and 50 diseased tubers which were, however, 21 cm. deep.

All the healthy tubers formed healthy plants; the 50 diseased tubers at 10 cm. depth only provided 24 plants, which were nearly all small and puny; some only were passably vigorous. It was ascertained that 18 of these plants were healthy, whilst 6 were diseased—a fact that was recognised by the presence of brownish streaks on the stems close to the earth. In some cases the brown streaks increased and spread to the lower leaves ("augmentaient et s'étendaient jusque dans les feuilles inférieures"). It was always easy to determine that they were produced by *P. infestans*, the mycelium of which had grown out from the tuber into the young shoots; on each plant spores of *P. infestans* were produced. By unearthing those tubers which had not come up, it was found that several bore shoots, which had not had sufficient strength to penetrate the earth because they had already been strongly attacked by the fungus. In some cases it was possible to induce a production of spores on these subterranean shoots.

Of the 50 diseased tubers planted at a depth of 21 cm. only 19 came through. In the case of 1 only, a single diseased shoot was observed on which spores were developed. This shoot died some days later. It was assumed that the greater depth of planting had prevented diseased shoots from reaching the surface.

All the potatoes were planted on May 11th; those at a depth of 10 cm. began to come through on the 26th and the others on the 31st. The first

shoot recognised as being diseased on the 29th was cut and placed under a bell-jar where it bore spores of the fungus. The remainder of the diseased shoots were found in the space of two to three weeks. Several died early without spreading the disease; they bore, however, spores close to the earth.

Observation on the spread of the disease from the affected shoots was kept and a detailed account is given. The conclusions were reached, firstly that the disease in the field has its place of origin in diseased tubers planted, the fungus growing from the parent tuber into the young shoots, secondly that the disease exists in the field as soon as the shoots have come through, although in a form unlikely to be noticed by the farmer, and thirdly that its increase is very slow at first because of the relatively small quantity of spores present but that it increases with growing rapidity as the spores multiply.

A watch was kept for the fungus in the neighbourhood of the town in gardens and in many fields but the disease did not start until after July 23rd, at which date diseased leaves were sought in vain. It was not until July 29th that some isolated spots of disease were found in most of the gardens and fields of the vicinity; that is, at a time when nearly all the leaves in the experimenter's garden were completely destroyed.

Two cases were observed where shoots which had not yet reached the surface were diseased, not at their base but about the middle, and it was concluded that such were examples of originally healthy shoots being attacked during growth by spores beneath the surface of the earth, possibly produced on a neighbouring part of the tuber. Jensen considers that shoots attacked in this manner are those which have a greater chance of reaching the surface and providing the sources of infection of the leaves. Those attacked *ab initio*, he considers, more often die beneath the surface but are nevertheless able to cause infection of other healthy shoots from the same tuber by contaminating the soil with spores produced on their surface.

To determine whether diseased shoots which had reached the surface bore spores at their base, suspected places were scratched with a knife and adhering particles were taken in a drop of water for examination with the microscope. It was found that spores were present but generally in small numbers. By means of applying a wetted finger to the brown coloured spots, spores were also successfully gathered.

Although the above experiments were uncontrolled to the extent that the diseased tubers were grown in unsterilised soil, the circumstances

under which they were conducted as well as the observations made, authorise, in our opinion, the acceptance of these experiments as well as those of de Bary as demonstrating that under certain conditions small diseased shoots infected *ab initio* and producing spores, arise from diseased tubers.

Dr Pethybridge (6) working in Ireland in 1910, obtained confirmation of the fact that naturally infected seed tubers may give rise to diseased shoots. The actual experiments were as follows: Six diseased tubers (variety "Champion"), chosen from a severely diseased crop, were halved, thus giving 12 diseased sets. As controls, 6 other healthy tubers of the same variety were similarly cut into 12 sets. Six diseased and 6 healthy sets were planted, on February 11th, in pots containing untreated virgin loam (not previously used for potting purposes) and the same number of diseased and healthy tubers, in similar loam previously sterilised. Six of the latter (3 diseased and 3 healthy) were placed, uncovered, in a warm greenhouse in an atmosphere fully charged with moisture. The remaining 6 pots containing sets in sterilised soil, as well as the 12 pots of the sets in unsterilised soil, were placed at the same time in an unheated greenhouse with an exceptionally dry atmosphere. No results were obtained from the plants in the heated greenhouse. Of the 18 sets in the unheated greenhouse, the 9 healthy sets produced healthy plants. Of the 9 diseased sets, 3 rotted in the soil. To quote the author's words: "The plants developing from the diseased sets were...somewhat less robust than those from the healthy ones, and one in particular...produced a shoot only 2 or 3 inches high which quickly became diseased from below upwards, and soon died. Doubtless the mycelium had entered the shoot from the parent set. This plant was removed as soon as possible for fear of its causing the infection of the neighbouring ones by 'spores[1].'" Up to April 27th all the plants, with the above exception, had remained healthy, but at this date, or immediately afterwards, spots of disease caused by *Phytophthora* were found on the leaves of 8 plants produced from healthy sets and of 4 plants from diseased sets. "Although absolute proof is lacking, it seems practically certain that the plants whose foliage became diseased must have become infected by means of 'spores' from

[1] This statement leaves it doubtful whether it was observed that spores were actually produced on this shoot. We may perhaps assume that this was the case, as elsewhere in the same paper (p. 15) the author has stated that "tubers which produce small diseased shoots above ground may be highly dangerous sources of infection, for on these shoots 'spores' are produced which can easily be carried to neighbouring healthy plants." It is not quite clear, however, whether the above statement refers to the author's own experiments or not.

the single diseased sprout sent above ground by one of the diseased sets[1]."

Prof. Melhus working in the United States, published in 1913 (4) and 1915 (5) the results of his study of the hibernation of the fungus. His experiments showed that, under favourable conditions, the mycelium in naturally infected potatoes readily passes out from the tuber into the developing shoots. Among the experiments, under controlled conditions in the greenhouse, in which naturally infected tubers produced shoots (infected *ab initio*) bearing conidiophores, the following may be mentioned:

(1) Twenty-four infected tubers (grown in three lots of 8 tubers each, at different temperatures) which were planted shallow (being only partially buried) gave rise, between 14 and 39 days after planting, to 23 infected shoots, on which spores of *P. infestans* were produced. These were described as "of all sizes, from those barely visible to those nearly 1 inch in length." This experiment was repeated twice, and spore-bearing diseased shoots were again obtained from some of the infected tubers.

(2) Nine infected tubers were grown, 6 in an air-temperature of 15–20° C., and 3 at 23–27° C., with controls. Forty-five days after

[1] Dr Pethybridge in his evident desire to point out the inherent improbability of, and the lack of scientific evidence for, the theory then recently put forward by Massee that potato plants commonly contain a latent mycelium which late in the season becomes dominant or not according to environmental conditions, appears to us to have been less than fair in his attitude to the evidence brought forward by earlier investigators as to the production early in the season of shoots diseased *ab initio*. Dr Pethybridge states: "The dormant mycelium theory is in reality but a modification of one brought forward in the first instance by de Bary, but discarded by him some five-and-thirty years ago as not being in accordance with known facts." Now de Bary in 1876 ((2) p. 267) summarised his earlier experiments, and after pointing out that, for some unknown reason, the fungus produced on diseased shoots coming from the diseased tubers did not spread to surrounding healthy plants, remarked "The negative result thus obtained caused me to doubt whether my previous explanation could hold good in the open field,...I accordingly repeated the experiment during the present year (1875)." These experiments (which we have summarised above, p. 290) gave, in de Bary's opinion, the fresh evidence that was needed and led him to reaccept the theory. What Dr Pethybridge quotes then as if it were the final opinion of de Bary was clearly only a temporary one. That this was so is evident from de Bary's own words later in the article ((2) p. 268): "...the problem before us is, as far as possible, solved; that is to say, I have shown that the oospores are not found in this district, and that the perennial mycelium discharges the function of hibernation which is proper to the oospores in other species." It is possible that the fact that in these later experiments de Bary used tubers artificially inoculated at the eyes and not naturally infected ones inclined Dr Pethybridge to regard these later experiments as beside the point, but there is no evidence, we submit, for his claim to quote de Bary as a partner in this view.

planting, the shoots of all the 6 plants at 15–20° C. were 7 in. tall and though kept under moist conditions they showed no signs of disease. In the case of the 3 tubers grown at 23–27° C. it was noticed after 39 days that one small shoot, about 2 in. high, appeared to be water-logged near soil level, and on its surface were found conidiophores and spores of *P. infestans*.

(3) Sixteen infected tubers were grown at 23–27° C.; 11 days after planting it was noticed that one shoot was just breaking through the soil. It appeared perfectly normal, but the next day "fungous growth" identified as *P. infestans* was found not only on this shoot but also on the surface of the soil immediately surrounding it.

(4) Twenty-four infected tubers were planted in steam-sterilised soil and the air temperature kept at 15–20° C. The soil was kept comparatively dry but after 30 days the tubers "were covered with a glass house and kept well watered." Thirteen days later a small spindly shoot showed discoloration at the surface of the soil and the following day the fungus fruited. Ten tubers rotted in the ground; the remaining 13 did not produce infected plants in the course of a further fortnight.

(5) Twelve "vigorously germinating" infected tubers were planted in slightly moist steam-sterilised sand. Two were dead after 12 days. The remaining 10, with sprouts that had broken through the surface of the sand, were potted in steam-sterilised soil and placed in a "glasshouse" where the soil was well watered and the air humidity high. Nine days later, one shoot was much discoloured near the surface of the soil, and after 2 days conidiophores of *P. infestans* were produced abundantly. Six days later another similar shoot was found. The remaining 8 tubers after a fortnight were found to be entirely decayed.

The above experiments were carried out under laboratory conditions. In 1913 and 1914 Prof. Melhus carried out investigations in the field.

In 1913, 256 whole infected tubers and 162 cut "seed" were planted in a field of potatoes on land that had not previously grown potatoes for at least 5 years. Thirty days after planting, 6 of the whole tubers which had failed to send shoots above ground were dug up. Four were dead and nearly decayed while the remaining 2 had 2 and 5 shoots, respectively, which were just ready to break through the surface of the soil. After these shoots had been placed in a moist chamber overnight, small patches of conidiophores bearing spores were found on their surface. On the 49th day a vigorous plant with 13 shoots, growing from a whole infected tuber was found to be diseased. One short stem, 6 in. long, was discoloured over a length of 1 in. and fungous growth with

spores of *P. infestans* was found. "Four other cases, similar to the one just described" of the production of diseased shoots by the remaining tubers are also mentioned as having occurred.

With regard to the cut "seed," it was noticed, 37 days after planting, that one stem was discoloured up to $\frac{1}{2}$ in. above the surface of the soil. On the next day a beaker was inverted over the plant, and the following morning the fungus was found to be fruiting.

In 1914, 3 cases of shoots infected *ab initio* were discovered in plants derived from 533 diseased sets in the course of a field experiment conducted in a manner similar to that adopted in 1913.

The experiments which we carried out at Wye during 1925 will now be described. The material used consisted of severely "blighted" potatoes, of the variety "Field Marshal," sent to us in February by a farmer. Three or four hundredweights of badly affected tubers had been picked out by this farmer from a two-ton "lot" of seed potatoes obtained from Scotland. On March 12th half a hundredweight of this material was examined by us. The tubers all showed the generally accepted external symptoms of blight, *e.g.* the skin was depressed and dark coloured, and in the majority of cases the dark areas extended nearly over the entire tuber. The appearance was somewhat remarkable, due to the fact that while the discoloured patches were exceptionally numerous, they had not inflicted sufficient injury to cause any rotting of the tuber to take place. Sections revealed the characteristic shallow brown areas of tissue lying immediately beneath the epidermis, and mycelium having been found in these, a number of thick slices of the entire tubers were placed in large covered glass dishes, at room temperature, without additional water, with the object of inducing growth of conidiophores and spores of the fungus.

Examination was not made at regular intervals, but on April 3rd abundant growth of conidiophores and spores was found.

It had been decided to plant 75 diseased tubers in the open and 25 in pots in a cold greenhouse and for this purpose the most affected "seed" was selected, thoroughly washed and placed in shallow boxes for sprouting in the light on the shelves of the unheated greenhouse.

For some time no growth took place and it seemed at first doubtful whether sprouts would ever develop on such badly diseased tubers. After about a fortnight, however, very small buds appeared on most of the tubers and growth continued slowly.

Early in April, 25 pots (9 in. diameter at the top) were prepared with

soil that had not previously been used for potatoes and one sprouted seed-potato was planted in each. These pots were placed in the unheated greenhouse and were watered when necessary. No attempt was made to control conditions of moisture or temperature[1].

About the middle of April, 75 diseased tubers were planted in the open and 50 of the same variety, selected as healthy, in a plot adjoining.

Inspection of the plants, either in the greenhouse or in the open, was not made at regular intervals, but on May 16th it was noticed that on each of 2 pot plants there was a short diseased shoot bearing two or three leaves already water-soaked and darkened. An examination by means of the microscope of a scraping from each of these shoots showed the presence of numerous conidiophores and spores of *Phytophthora infestans*. These plants were immediately removed from the greenhouse. The remaining 21 plants (two of the original tubers planted had made no growth) were closely inspected and no trace of the fungus could be found. All had made vigorous growth, each having several shoots up to 8 in. in height with perfectly healthy leaves. Of the 2 plants producing diseased shoots, the first had only three stems; one, $4\frac{1}{2}$ in. long with four leaves and terminal bud; another, 1 in. long with a bud but no expanded leaves, and the last, 1 in. long with two leaves and a terminal bud. The last-mentioned shoot was the only one showing signs of disease; its lower leaf was black and dead and the upper was covered with conidiophores. This leaf was 2 in. long and was curled downwards close to the earth.

The second plant (Plate V, figs. 1 and 2) was rather larger, having five shoots: (*a*) $7\frac{1}{4}$ in. high with six leaves—the lowest spathulate and the

[1] The following are records of the air temperature in a Stevenson screen in the open at Wye during March, April and May. During the day the greenhouse was well ventilated both by door and windows but at night it was closed.

Air Temperature at Wye, Kent, in degrees Fahrenheit.

	1925		
	March	April	May
Mean temperature at 9 a.m.	41·0	47·9	56·0
,, ,, at 9 p.m.	38·6	43·4	51·6
,, minimum (night) temperature	34·7	37·2	46·0
,, maximum (day) ,,	46·1	53·2	61·6
Highest maximum temperature	52·0	60·0	75·0
Lowest minimum ,,	26·0	27·0	36·0
Hours of sunshine	103·0	120·6	220·5
,, ,, mean per day	3·3	4·1	7·1

These figures are from records kept by Mr A. H. Bird, M.A., in connection with the agricultural meteorological scheme of the Ministry of Agriculture.

rest divided. The second leaf from the ground bore, on its lower surface, an isolated water-soaked area with whitish margin. Conidiophores and spores of *Phytophthora* were found on this and it was assumed to be a case of secondary infection probably originating from the near-by dwarf shoot (*e*) described below. (*b*) 7 in. high, having five leaves and a terminal bud. The lowest leaf was spathulate, the rest divided, and all were healthy. (*c*) $7\frac{3}{4}$ in. high, having six leaves and a terminal bud. The lowest two leaves were spathulate, the rest divided and all were healthy. (*d*) $3\frac{3}{4}$ in. high with five small leaves, the lowest of which was dead and the next one bearing conidiophores and spores of the fungus. The stem was thin and spindly and was streaked with brown marks. No attempt was made to determine whether these marks were due to the presence of the fungus. From its appearance, this shoot might have been the most recently produced and might well have become infected on both leaves and stem by spores derived from the dwarf shoot (*e*) described below. On the other hand, it might possibly have been an attenuated shoot infected by mycelium from the tuber. (*e*) 1 in. high, having three small leaves, two of which were brown, wet and dead, and the third covered with conidiophores bearing spores of *Phytophthora* (see Plate V, fig. 2). The tip of the shoot (terminal bud) was already wet and shrivelling.

In the case of the 2 plants described above, external evidence alone was relied upon and no microscopical examination was made to trace the course of any mycelium existing in the tuber and shoot.

The only possible sources of infection by the fungus at a date earlier than May 16th would be either oospores resting in the soil or mycelium which had grown up inside the shoots of the planted tubers and of these the latter is the more probable for the following reasons. Firstly, oospores of *Phytophthora infestans* are not known to occur in nature[1], secondly the soil used for the experiments had not previously grown potatoes, and thirdly the remaining 21 plants under the same conditions had continued perfectly healthy.

As regards the possibility of infection by conidia from some outside

[1] In a paper by Prof. Melhus(5) the statement is made that "... *P. infestans* rarely produces oospores in the potato plant...." In reply to an enquiry, Prof. Melhus has written to us (Dec. 16, 1925): "I know of no one who has actually obtained such data but I do know of several perfectly good pathologists who have from time to time thought they found oospores.... You should bear in mind that I advanced these findings close on the heels of Clinton, Jones and Pethybridge's discoveries of oospore production in pure culture. I could hardly neglect entirely the inference implied in their work, and of course it may well be that oospores actually are produced under certain conditions sometimes."

source, the early date (May 16th) would seem to preclude any field or garden crops being held responsible[1]; moreover, if such source existed it might be expected that more than 2 amongst 23 plants would have become infected, whereas, after removal of the 2 plants described, all the 21 remained free from any trace of disease for a further $2\frac{1}{2}$ months when they were finally dispensed with as controls[2].

No useful results were obtained from the plants grown in the open. All the 50 derived from healthy tubers were free from disease up to July 30th. Of the 75 diseased tubers originally planted, only 40 had produced above-ground shoots and these 40 were all free from *Phytophthora* on July 30th. It was not found possible to examine the plots again until August 16th, when it was observed that spots of blight were of common occurrence on the leaves of plants in both plots. At this date, however, secondary infection such as this was probably general in the neighbourhood.

The theory of the method of perpetuation of the disease, by means of an infected shoot arising from a diseased tuber, so clearly put forward by de Bary in 1861, may, we consider, be now looked upon as finally established beyond doubt, chiefly through the thorough and careful investigations carried out by Prof. Melhus. The infected shoots obtained in our experiment and described above were closely similar to those observed by previous investigators.

Summary.

(1) A résumé is given of the work of those investigators who have recorded the occurrence of diseased shoots arising from potato tubers infected by *P. infestans*.

(2) An experiment is recorded in which of 25 naturally infected tubers planted early in April in pots in the greenhouse, 2 produced by May 16th short diseased shoots, which developed conidiophores and spores. These shoots, doubtless infected *ab initio* by mycelium growing from the tuber, were closely similar to those which have been described by de Bary and other investigators.

(3) A probable case of secondary infection was observed on a leaf on one of the healthy shoots of a plant which produced a diseased shoot.

[1] It was not until August that the occurrence of potato blight in the open was recorded in the S.E. of England; at Wye it was first observed on the 16th of that month.

[2] On July 30th these plants were destroyed as being no longer required.

EXPLANATION OF PLATE V.

Fig. 1. Potato plant (var. "Field Marshal") grown from a diseased tuber. Of the five shoots, the shortest (at *e*) has probably been infected by mycelium from the parent tuber. It was covered with conidiophores and spores of *P. infestans* and was starting to decay. On a lower leaf of another shoot (*a*), there was an isolated area of disease —probably due to secondary infection. Brown marks were observed on the stem of another shoot (*d*). The rest of the plant (*b* and *c*) showed no signs of disease. Planted early in April. Photographed May 17th, 1925. $\frac{5}{12}$ natural size.

Fig. 2. A part of the plant illustrated in Fig. 1. In both illustrations, it will be noticed that the covering earth has been washed from the tuber after the latter had been removed from the pot. $\frac{5}{6}$ natural size.

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(*Received December 17th*, 1925.)

Fig. 2.

Fig. 1.

SALMON & WARE.—Occurrence of Diseased Shoots from Potato. (pp. 289–300.)

FURTHER EXPERIMENTS ON THE USE OF SULPHUR IN RELATION TO WART DISEASE OF POTATOES

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A. Introduction.

In a former communication(1) experiments were described the object of which was the elimination of the fungus causing Wart Disease (*Synchytrium endobioticum*) from contaminated soil. In the course of this work attention became focussed on sulphur because of its apparent efficiency as a fungicide and of its cheapness. Results at Ormskirk in 1924 showed that for applications of sulphur not exceeding about 10 cwts. per acre, the reduction in the amount of disease in the crop of potatoes immediately following the treatment was proportional to the amount of sulphur added. A consideration of these results alone leads to the tentative conclusion that an application of 11·2 cwts. of sulphur per acre should be sufficient to render the soil able to bear a crop quite free from disease. Even with an application of 20 cwts. of sulphur per acre, however, there was a small amount of disease remaining. It was suggested that this residual infection might be due to re-contamination from the outside by such agencies as wind, vermin, etc., after the fungicidal action of the sulphur was finished.

The experiments about to be described were designed to provide more data on the effect of the treatment on the disease, and, in particular, to test the above hypothesis put forward to explain the residual infection with the 20 cwt. dressing. Further, had the treatment resulted in a complete, or almost complete, absence of disease in the crop immediately following, it was intended to continue the plots with a susceptible variety of potato during one or more succeeding years to determine,

firstly, whether the effects of the treatment were permanent, and, secondly, to what extent, if any, the treatment affected the soil. As will be seen, the results did not justify this extension of the experiment.

B. Ormskirk Experiments.

(1) *Arrangement of Plots.* The experiment was carried out in the grounds of the Ormskirk Poor Law Institution. In the previous year the field had carried a good crop of oats, and just before the treatment was commenced the area to be experimented on was given a liberal dressing of pig manure. The following arrangement of plots was adopted; each plot measuring 90 × 29 ft.

Table I.

Plot no.	Treatment
1	None
2	15 cwts. sulphur per acre in November
3	10 „ „ „ „
4	7½ „ „ „ „
	7½ „ „ „ March
5	5 „ „ „ November
	5 „ „ „ March
6	10 „ „ „ „
7	15 „ „ „ „
8	None

(2) *Application of the Sulphur.* To ensure even distribution of the sulphur each experimental plot was divided up into ten equal areas. The sulphur was weighed out in 3 or 4 lb. lots and the requisite number of these placed on each small area. The sulphur was applied by means of a sieve (about 10 meshes to 1 in.), the ground being covered more than once so that any inequalities of distribution should be levelled as far as possible.

The treatment was carried out severally, in the autumn, in the spring and in both autumn and spring in order to obtain preliminary data on the variation, if any, in the effect of the treatment according to the time of the year.

The sulphur was incorporated by means of the Simar Rotary Tiller on plots 2, 3, 4 and 5 during November 13th to 17th, 1924, and on plots 4, 5, 6 and 7 during March 19th to 22nd, no work being done on the 21st owing to heavy rain, which rendered work on the soil impossible. On both occasions treatment was postponed as long as possible in the hope that a period of fine weather would occur; but finally it had to be

done a short time after the rain had stopped. The soil, therefore, was not in a state so conducive to extremely thorough mixing with the Simar machine, as it had been in previous years, but the incorporation was far more thorough than would result from any more ordinary cultural operation.

(3) *Fencing.* The fencing in of plots 2 and 3 was unavoidably delayed for a few days, but was finished by November 29th. As the soil was wet during the whole of the time that it was exposed (the rain commenced half an hour after the cultivation was completed) it is unlikely that the plots became contaminated by wind-blown sporangia at this time. As there was no large track on the plots, re-contamination by vermin, etc., is probably excluded, but various birds commonly flew from untreated to treated plots and conversely. Owing to illness the fencing of the plots 4, 5, 6 and 7 was not completed till the end of May. The fencing, to a height of 4 ft., consisted of brattice cloth nailed to battens at the top and bottom, and, above this, 2 ft. of wire-netting. The experimental area was on the protected eastern side of a wall about 8 ft. high, running approximately north and south. Even with this protection from the prevailing wind a certain amount of damage was done to the fencing by the heavy gales experienced in the district during the winter of 1924–5. As the damage to the external fence was on the eastern side during a gale from the west, the soil being saturated at the time, little if any contamination is likely to have taken place as a result.

(4) *Planting of Potatoes.* King Edward potatoes were set on May 7th–9th, but they failed to grow. The whole surface of the treated plots was sprayed with dilute formaldehyde in the hope of killing any sporangia which might have been accidentally blown or carried in, and a second planting was made on July 1st–2nd, Arran Chief potatoes being used. The few plants resulting from the first setting were left.

(5) *Effect of Treatment on the Crop.* Both settings failed almost entirely to grow, comparatively few shoots exceeding 3 in. in height. A few plants, however, on each plot made fairly satisfactory growth. In many cases the tubers had a rough brown skin, being obviously burned by the sulphur in the soil.

(6) *The Effect of the Treatment on the Disease.* Every plant on the treated plots, which was considered sufficiently well developed to show Wart Disease if growing in contaminated soil, was lifted and examined. As the control plots in which the plants grew well were obviously heavily infected only a few plants taken at random were lifted for examination. The results were as follows:

Table II.

Results of Ormskirk Experiment 1925. The percentage numbers of warted and clean plants are only very approximate.

Plot no.	Treatment	Plants examined	% warted	% clean
1	Control	20	100	0
2	15 cwts. S.	29	28	72
3	10 „ S.	93	42	58
4	7½ and 7½ cwts. S.	10	[10]	[90]
5	5 and 5 cwts. S.	4	[50]	[50]
6	10 cwts. S.	28	39	61
7	15 „ S.	12	42	58
8	Control	50	100	0

The disease on the plants in the treated plots tended to occur at the level of the surface of the soil; on the plants in the untreated plots it was more evenly distributed. Both the above figures, and the impressions gathered from the inspection of the plots, lead to the conclusion that a reduction in the amount of disease was brought about by the treatment. The amount of reduction, however, was smaller than that obtained in 1924 and in earlier experiments. The experiment, therefore, failed in its main purpose, namely, the attempted production of an area quite free from disease.

C. Hatfield Experiments.

The experimental area at Hatfield was the one used in the work described in a previous paper(1) and with the exception of certain small portions, the soil had already been treated with varying amounts of sulphur. In 1924 an incorporation of 2 tons of sulphur per acre had produced a stunted but clean crop.

(1) *Experimental Details.* An attempt was made to free entirely the whole experimental plot of disease. Ground sulphur was applied at the rate of 3 tons per acre and incorporated by means of the Simar machine on May 15th. The degree of incorporation here was even less satisfactory than at Ormskirk: the heavy clay soil had formed at the surface a hard crust, which broke into lumps that were difficult to reduce to a fine state of division.

King Edward potatoes were planted on June 25th and 26th.

(2) *The Effect of the Treatment on the Crop.* On particular areas of the plot which, in previous years, had received no sulphur, the application in 1925 of 3 tons per acre of sulphur had little effect on the crop. The foliage appeared quite normal, but the surface of some of the young tubers was brown and rough. Elsewhere, however, on areas which had

received any considerable quantities of sulphur in previous years, there was practically no growth of the potato sets.

(3) *The Effect of the Treatment on the Disease.* On the areas of the plot receiving sulphur for the first time there was a considerable amount of Wart Disease. Whether, however, the treatment had had any effect on the disease in the present season it was impossible to ascertain in the absence of untreated plots.

D. General Conclusions.

(*a*) *Effect on Disease.* The effect of sulphur treatment of soil on the incidence of Wart Disease in the first crop of potatoes has been as follows in our field experiments:

At Ormskirk on a sandy-peat soil.

1922 and 1923. Indications that sulphur considerably reduced the amount of disease.

1924. In a year of heavy infection, the amount of disease was reduced from 73 to 8 per cent. in proportion to the amount of sulphur added up to a treatment of 10 cwts. of sulphur per acre. One ton of sulphur per acre reduced the amount of disease to 4 per cent.

1925. A reduction of disease in a year of heavy infection.

At Hatfield on heavy clay soil.

1923. A great reduction of disease in a year of heavy infection.

1924. A complete absence of disease in a year of heavy infection.

1925. Little, if any reduction in the amount of disease in a year of heavy infection.

Both at Ormskirk and at Hatfield, the effect of the treatment on the disease was less in 1925 than in previous seasons. The results up to 1924 inclusive indicate that, under the experimental conditions of those years, sulphur treatment, at the rate of 1 ton per acre or less at Ormskirk and of 2 tons per acre at Hatfield, greatly reduced disease in the succeeding crop of susceptible potatoes. Under the experimental conditions of 1925 a reduction in the amount of the disease was produced at Ormskirk by treatment with 10 and 15 cwts. sulphur per acre.

(*b*) *Effect on Crop.* The variation in the effect of the treatment on the potato crop immediately following is great. The results may be summarised as follows:

(1) 1922 Ormskirk (light soil). Potatoes set three weeks after treatment. Tubers badly "scorched" and cracked, otherwise crop not affected by an application of 20 cwts. of sulphur per acre.

(2) 1923. Potatoes planted immediately after the incorporation of sulphur.

Ormskirk (light soil). Crop badly stunted and tubers "scorched" by 20 cwts. sulphur per acre.

Hatfield (clay soil). Crop little affected by 20 cwts. sulphur per acre. With 40 cwts. sulphur per acre a reduction in foliage and in crop, the tubers having rough brown skins.

(3) 1924. Potatoes planted six weeks after sulphur treatment.

Ormskirk (light soil). Plants stunted by 20 cwts. of sulphur per acre.

Hatfield (clay soil). Crop "scorched" by 20 cwts. of sulphur per acre, and badly so by 40 cwts. per acre.

(4) 1925 Ormskirk (light soil). Potatoes set at least seven weeks after treatment. Almost complete failure of crop with 10 or 15 cwts. sulphur per acre.

Hatfield (clay soil). Potatoes planted six weeks after treatment. Crop hardly affected by 60 cwts. sulphur per acre.

E. Degree of Permanence of the Effect of the Treatment on the Crop.

The Ormskirk plots which were treated with sulphur in 1924 (1) were, in 1925, given a uniform dressing of lime at the rate of about 1 ton per acre and the inside half of each plot received an additional dressing of powdered limestone at the rate of 2 tons per acre. In every other respect the area was treated in the normal commercial manner. Majestic (immune) potatoes were grown. The crop was good, even for the district, and it was impossible to pick out by the inspection of the plants in the field the plots treated with sulphur in 1924 from the untreated plots. The potatoes were dug from the three plots which in 1924 had received 20 cwts. of sulphur per acre and were adjacent to the plots which had received no sulphur. There was no significant difference between the produce of the two plots in any of the three cases. In this experiment, therefore, the effect of the treatment on the crop was only temporary and could not be distinguished in the following season. Further, it has not been found possible to pick out plots used by us in previous years by any residual effect of the treatment on the crops grown in succeeding years, so that the absence of any permanent adverse effect seems to be generally true. The crops taken in 1926 from the area treated in 1925 will be examined.

Summary.

Plots of light sandy soil at Ormskirk carefully fenced in to prevent re-contamination were treated with sulphur at rates of 10 cwts. and 15 cwts. per acre. The sulphur was incorporated by means of the Simar Rotary Tiller. A planting of King Edward and a replanting of Arran Chief varieties in the treated plots almost completely failed to grow. On the few plants which developed, Wart Disease was present in less quantity than on the plants in the untreated controls.

A plot of heavy clay soil at Hatfield was treated with 3 tons of sulphur per acre incorporated by means of the Simar Rotary Tiller. Plants of King Edward variety grew well but showed a considerable amount of Wart Disease.

Plots of land at Ormskirk which in 1924 had received amounts of sulphur varying up to one ton per acre were in 1925 given a dressing of lime and planted with Majestic variety of potatoes. No effect of the previous treatment on the crop was apparent.

We are indebted to the Piccard Pictet Company for the loan of the 4 h.p. Simar Rotary Tiller used in these experiments.

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(*Received January 3rd*, 1926.)

DISCUSSION ON "THE FUNGICIDAL ACTION OF SULPHUR"

ORDINARY MEETING. *OCTOBER* 20, 1925.

Professor E. S. Salmon (Wye College) in opening the discussion said: The investigations on the fungicidal value of sulphur carried out at Wye College have been chiefly concerned with the action of certain polysulphides—viz. those of ammonium, calcium and potassium. The chemical aspect of these investigations will be dealt with by my colleague Dr Goodwin.

There are a few observations on the biological side which I should like to lay before this Meeting.

Firstly, may I be allowed to mention a certain experiment—a very simple and primitive one—with regard to the action of sulphur made many years ago? In Kent (as in all the hop-growing counties) farmers are in the habit of dusting the hop-plant each season with flowers of sulphur to control "mould" (*Sphaerotheca Humuli*). Hundreds of tons of sulphur are used in this way in Kent alone. The question was frequently asked me—How does sulphur act in killing or preventing "mould"?—followed up usually by the question—Is a high temperature necessary, such as obtains in the sunshine on hot days in summer? The point raised was of course not an academic one, but of practical importance—for if a high temperature were necessary for the fungicidal action of flowers of sulphur, then in the spells of cool, wet, dull weather which are so common in English summers it would be preferable to use—as several hop-growers had already concluded—a fluid spray, viz. a solution of liver of sulphur. The farmers told me that on hot days the sulphured hop-gardens gave out a strong smell of sulphur and the more imaginative of my farmer friends assure me that they had seen on such occasions a distinct blueish vapour hanging in the hop-garden!

Now in order to try to answer the question of what conditions were necessary *in the field* for flowers of sulphur to exert its fungicidal properties, I turned first to the technical literature. And, as so often happens, the more I read, the greater the confusion grew. Every theory had been put forward, often with considerable emphasis; there was no doubt at all how sulphur acted, *but* each explanation was a different one! Dogmatic statements were printed (*e.g.* by Marshall Ward) that the sulphur dioxide evolved was the efficacious agent—while other theorists held that sulphuric acid was formed or that temperatures were reached in the open which caused volatilisation. And I felt it difficult to decide in my mind who had added most to the general confusion—the chemist working without the assistance of a mycologist, or the mycologist without the chemist.

Then it occurred to me that a very simple experiment would determine whether actual sunshine was necessary for sulphur to exert its fungicidal power.

I arranged under the microscope a young hop-plant infested with mildew in such a way that I could slightly move it, pot and all, according as it was necessary to keep any patch of mildew on its leaves focussed under the microscope. I took a

very fine glass hair and using a low objective placed a very minute fragment of pure "flowers of sulphur" on the terminal conidium of a chain of conidia. The plant was kept on a bench in the laboratory where no sun reached it. Under these conditions the sulphur exerted a very slow fungicidal action, leading after 3 days to the killing of the chain of conidia. The first signs of the death of the conidium was a slight shrivelling and the appearance of striae on the wall. Only the conidiophore which actually bore the sulphur particle was affected—other conidiophores in the closest proximity—only $\frac{1}{800}$ in. or 30μ away—remained healthy and growing during the time of the experiment.

The experiment showed therefore that sunshine is not necessary—and I was able to answer therefore the farmers' questions on that point. I concluded also—rightly or wrongly—that the flowers of sulphur under these conditions was not acting in any gaseous form—as the closely adjacent conidiophores remained unaffected; also that actual contact of the sulphur particles with the fungus is necessary for the fungicidal action to take place. If it is really a fact that in the hop-garden flowers of sulphur does not control mildew in dull and wet summers, the explanation probably is that the rain washes off at any rate the bigger particles of the flowers of sulphur from the mildew, and of course from the healthy parts of the leaf where it is applied as a preventive.

With regard to the fungicidal action of polysulphide, our main observations have been chemical, and these will be mentioned by Dr Goodwin.

There are, however, a few observations on the biological aspect which I should like to make, although perhaps some of them are a little wide of the immediate question of the action of sulphur.

We found that the lethal doses of various solutions containing polysulphides could be accurately determined and compared with one another if we "standardised" both the host-plant and the mildew.

Taking the mildew first—it might have been thought that the very early stage after germination of the conidium, *i.e.* before the sporeling plant has formed conidiophores and conidia, would be easier to kill than the later stage when the mildew forms "powdery" patches bearing conidiophores and chains of conidia. As a matter of fact, the reverse is the case—the early stage requiring approximately a solution of double the concentration lethal for the later stage.

Then we found that a powdery patch of mildew on an older leaf of the hop-plant was easier to kill than the same stage on a young actively growing leaf. Finally we chose for comparative experiments the powdery stage of the mildew growing on young, vigorous leaves. To avoid variation as far as possible due to the effect of different host-plants, we used clone plants of one susceptible variety of hop.

The visible fungicidal effect of all the solutions of polysulphides used was the same and was very different from that observable in the case of sulphur. As soon as the solution applied has dried—and this takes place in a few minutes—it can be seen that all the conidiophores have collapsed; the mycelium although still white and not shrivelled up has an abnormal floccose appearance.

If the solution is of lethal concentration, the dead mildew remains white and unchanged for 2 or 3 weeks—if the solution is below the necessary strength, the mycelium after remaining sterile for a day or two, revives and begins to produce fresh conidiophores.

These observations led us to the conclusion that the various polysulphides act fungicidally as such, although it is of course possible that they do so through the sulphur deposited by their decomposition.

Dr Goodwin (Wye College) said that some ten years ago Eyre and Salmon had studied the action of liver of sulphur and ammonium polysulphide on powdery mildews and had found in the case of liver of sulphur that the fungicidal action was not due to free alkali. Also that the proportion of sulphide sulphur was not an index of the fungicidal value of solutions of alkaline sulphides. In the case of ammonium polysulphide particularly it seemed clear that it was the polysulphides in the solution that acted fungicidally.

Further work in this field confirmed the view that in polysulphide-containing sprays the polysulphides from different sources behaved in the same manner as fungicides against the hop powdery mildew, the results being the same whether liver of sulphur, lime sulphur or ammonium polysulphide were used—of course at equivalent strengths.

It thus became necessary to survey the methods of analysis used in the determination of polysulphides so that a strict comparison could be made of the polysulphides in the various spray fluids. The method recommended by R. M. Chapin was found, after slight modification had been made, to give very satisfactory results with the three main polysulphide sprays and this method has since been used to determine the strength of the solutions used in the experiments that have been undertaken.

In collaboration with Professor Salmon, biological tests with the hop mildew were carried out with a number of sprays containing polysulphides and it was found that liver of sulphur at a strength of 0·11 per cent. polysulphide sulphur was fungicidal whereas at 0·066 per cent. the effect was nil or very slight, other tests gave 0·12 per cent. and 0·092 per cent. as fungicidal, so that 0·1 per cent. polysulphide sulphur may be taken as the effective strength. This figure agrees with 0·11 per cent. found by Horton and Salmon in the case of lime sulphur. A trial was also made with a sodium liver of sulphur and at 0·12 per cent. polysulphide sulphur it was found to be definitely fungicidal but not at lower strengths. It may be of interest to mention that the makers of this last preparation recommended that it should be used at a strength which was found to be equivalent to only 0·25 per cent. polysulphide sulphur. At this strength the material was absolutely worthless.

The agreement shown in the figures just mentioned seems to show clearly that the polysulphides, whether in liver of sulphur, lime sulphur or sodium polysulphide, act in the same way. This conclusion does not of course settle the question as to how exactly the polysulphides act, but it has established the point which was in view when the work was started.

A further series of biological tests were carried out to ascertain whether the admixture of lead arsenate with lime sulphur exercises an adverse effect upon the polysulphides in the spray. The results have not yet been published and they require confirmation, but from them and from the thorough chemical examination of the mixed spray the polysulphide sulphur does not seem to be altered to any important extent by the addition of the lead arsenate but to exercise the same effect as in the original unmixed solution.

There is very strong evidence from the chemical side that the water-soluble arsenic in the mixed spray has considerable fungicidal properties and that it becomes an important factor in the case. This view finds support in the biological tests. A further question which may have economic consequences is whether by mixing the lime sulphur with lead arsenate the former can be used at much lower strengths; experiments in this connection are in prospect.

Professor B. T. P. Barker (Long Ashton) said: The following account deals with an investigation on the mode of action of sulphur as a fungicide which has been carried on at Long Ashton since 1919. The work is still incomplete, but in certain directions results of a definite character have been achieved.

Messrs C. T. Gimingham and S. P. Wiltshire, prior to their departure from Long Ashton, were collaborators in the earlier stages of the investigation. The later experiments have been mainly carried out by Mr W. Camps, the senior research assistant at that station, who has throughout been associated with the work.

At the outset it was recognised that attention could not be confined to the action of uncombined sulphur. Hence the action of spray fluids of the polysulphide type was examined and the conclusion arrived at that their last protective fungicidal action must be attributed to the sulphur which is rapidly produced by the decomposition of the polysulphides after the application of those sprays to the plant.

The investigation thus became reduced to the single issue of the toxicity of sulphur itself. A series of experiments of a varied type demonstrated that the toxic effect could be obtained across a space: that is to say, actively growing fungi could be killed at some distance from the actual place of application of the sulphur.

The manner of this action of sulphur "at a distance" has been studied and the following experiments appear definitely to disprove the agency of any substance in gaseous form.

A coating of sulphur was applied to the hot water pipes of a greenhouse and a number of young wheat plants attacked by *Erisyphe graminis* were brought into the house, controls being left in an adjacent house. The fungus was killed in the former case, but remained vigorous in the latter. Further plants were then introduced into the house under closed bell jars so arranged that a current of the greenhouse air could be drawn continuously through. In one set the air was filtered through a cellulose pad before entering the jars, while in the remainder it was unfiltered. The mildew remained living in the former, but was killed in the latter. Examination of the filter pads showed the presence of very finely divided sulphur. A similar result was obtained without filtration when the aspirated air was first passed through a bent tube, sulphur being deposited at the angle of the tube. Using bright copper wire in the place of the wheat plants, a corresponding result occurred, copper sulphide being formed when the air was not deprived of its sulphur content by filtration or deposition.

These results are difficult to interpret except by the hypothesis that the toxic agent is travelling in the form of a solid, rather than a gas or vapour. It is certain that the substance as a solid is in an extremely fine state of division, since it requires a very fine filter to remove it from the atmosphere. Analogous cases are known and the substances concerned are described then as being in particulate condition. It can thus be concluded that sulphur is capable of being dispersed over material distances

under suitable conditions in particulate form. All the evidence available indicates that the particulate matter in this case is sulphur itself and not a compound.

There is further evidence available of the existence of sulphur in this particulate form. Glass slides exposed in a greenhouse treated with sulphur as above, or in a chamber in which a cloud of sulphur has been put up by a Campbell vaporiser become covered with a deposit of microscopic sulphur particles. Similarly, if black-currant shoots carrying buds affected with bud mite and about to start into growth are allowed to stand in a sulphur-charged atmosphere of this description for a few hours and are then removed into a sulphur-free room, the mites are killed on attempting to migrate from the buds as they open, death being caused by the particulate sulphur adherent to the bud scales and surface of the shoot.

From the applied point of view, some knowledge of the conditions under which a large yield of particulate sulphur can be produced is important. The cloud of sulphur produced by the Campbell sulphur vaporiser is composed of particles of various sizes formed by the condensation of the vapour given off by the boiling sulphur.

A method described by Vogt giving a large yield of particulate sulphur consists in passing a current of steam over molten sulphur. Wallace and the present author have devised an effective and somewhat similar method in which a current of air is used in place of steam and the operation is conducted at a much lower temperature than in Vogt's method.

In each of these cases the sulphur is heated in the first place to a relatively high temperature. What happens under ordinary conditions in the field has not yet been fully investigated, but the following experiment indicated that the process may be complex.

When a current of air is passed through a suspension of flowers of sulphur in hot water slightly acidulated with sulphuric acid no visible particulate sulphur is given off and a similar negative result follows when carbon dioxide is subsequently blown through. When, however, after passage of carbon dioxide for about 15 minutes air is again passed, comparatively dense clouds of particulate sulphur are discharged.

Thus the action of sulphur at a distance has in part been explained. How sulphur can be dispersed to reach portions of plants not directly touched by a sulphur spray or powder and how it can come into actual contact with the fungus spore or mycelium is now clear. It still remains to be proved how it acts as a toxin. There seem to be three alternative views which require examination: and work in connection with these is still in progress.

The first possibility is that the particulate sulphur is dissolved in the film of moisture covering the fungus. Simple solution appears to be disproved by the fact that attempts to dissolve sulphur in water have yielded a liquid of non-toxic properties.

The second alternative—one suggested by analogous cases of other substances in particulate state—that sulphur is directly toxic in its particulate state, is so far unsupported by positive evidence.

The third view is that a sulphur compound is formed from the particulate material.

Under the latter view the various substances which for various reasons require consideration are sulphur dioxide and trioxide, sulphuric acid, pentathionic acid and sulphuretted hydrogen and compounds of the sulphide type.

Of these there has been obtained no evidence of formation in adequate strength of sulphur dioxide, trioxide and sulphuric acid.

The view that pentathionic acid was formed and acted as the toxic substance was put forward by Young. No evidence of its formation has been obtained during the course of the Long Ashton work, although it has been repeatedly sought. Moreover, Young has produced no evidence of its toxicity or volatility and his statement that it suffers decomposition in solutions outside limits of *p*H 4·2–5·4 taken in conjunction with his result of fungicidal activity of sulphur below *p*H 3·2 and 2·4 seems to put it definitely out of account.

The position with regard to sulphuretted hydrogen and compounds of the sulphide type is more open. When sulphur has been sprinkled on living leaves attached to the parent plant the production of small amounts of a volatile substance giving the reactions of sulphuretted hydrogen, as shown by the blackening of metallic silver and lead acetate paper, has been demonstrated. This is much less in the case of leaves removed from the plant: and no reaction has been obtained from a mixture of sulphur and the crushed leaf tissue. Also when a film of water is allowed to stand for some hours on a living leaf it can dissolve a small quantity of sulphur—apparently as the result of chemical action—for sulphuretted hydrogen is given off on treatment with nascent hydrogen. Further investigation of the relations between the living leaf and sulphur is in progress.

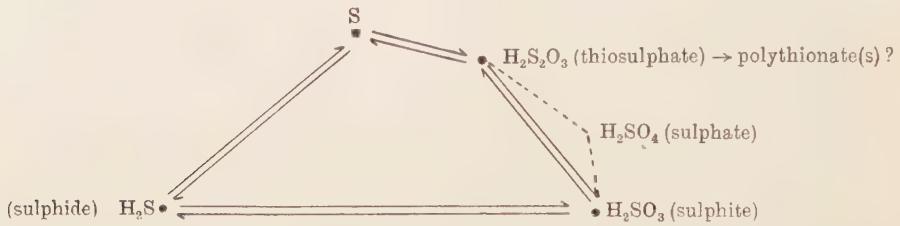
So far in the course of these experiments the latter are the only sulphur derivatives of possible toxic significance of which positive evidence of formation has been obtained. H_2S is not generally accepted as a factor in the fungicidal property of S partly because its presence has not been hitherto demonstrated and partly because its fungicidal action had not been conclusively proved. The former difficulty has not been removed, but the conflict of evidence on the latter point still remains.

Mr F. S. Aumonier said that Dr Goodwin's modification of the Chapin method for estimating polysulphides in lime-sulphur solution had been used at the Government Laboratory. In his opinion it was a good method and should be capable of giving satisfactory results in the hands of Works Chemists. He thought perhaps sulphur vapour might account for some of the fungicidal action attributed by Professor Barker to "particulate" sulphur. He also suggested that in the experiments designed to detect sulphur compounds possibly formed when sulphur is in contact with leaves, the failure to obtain evidence of any compounds other than sulphuretted hydrogen might be due to the fact that for this substance a very sensitive test, viz. the lead acetate test, was available, whereas for sulphur dioxide and for pentathionic acid the methods of detection used were probably less sensitive.

Professor Barker in reply stated that the greenhouse experiments referred to in his earlier remarks seemed definitely to disprove the suggestion that the toxic substance was sulphur vapour. The toxic properties of the sulphured greenhouse atmosphere were removed both by its filtration and its passage through a suitably bent tube. With regard to the action of living leaves upon sulphur he was not prepared at present to commit himself to any more positive statement as to sulphur dioxide and pentathionic acid than that no evidence of their production was shown by any of the standard tests.

Mr W. A. Roach (Rothamsted Experimental Station) said: So far the meeting

has discussed the fungicidal action of sulphur and some of its compounds when applied directly to the plant or to the fungus as a spray or dust; I would like to indicate a few of the complications which arise when its action takes place in the soil. There is evidence that under aerobic conditions sulphur in the soil is oxidised through thiosulphate to sulphate, and to a substance or substances unaffected by iodine, possibly one or more of the polythionates, and that under anaerobic conditions it is reduced to sulphide. The formation of sulphite and other compounds is quite possible.



These changes may be brought about by micro-organisms and some of them by the soil without intervention of micro-organisms; some are reversible and all are influenced by soil conditions. It is not surprising then that the fungicidal action of sulphur and its compounds in the soil is different in certain respects from its action when applied directly to the plant and that its action varies somewhat from year to year and from soil to soil.

Dr Goodwin has just said that the fungicidal actions of ammonium, calcium and potassium polysulphides are practically the same, yet, when used against wart disease in the soil in our experiments, ammonium polysulphide caused no reduction of the disease, the calcium compound may have caused a slight reduction, but the potassium compound caused a very marked reduction in the disease. The variation of efficiency of the sulphur treatment from soil to soil is illustrated by the fact that at least twice as much sulphur is needed on heavy clay soil as on light peaty soil to produce equal effects on the disease. The fact that this year the treatment has been definitely less effective than in the previous four years suggests that soil conditions other than those dependent on soil type play a part. The effect of these varying conditions on the changes undergone by sulphur in the soil is even better demonstrated by the effect of the treatment on the potato:

1923. Potatoes planted immediately after the incorporation of the sulphur.
Light Soil—crop badly "scorched" by 20 cwts. per acre sulphur.
Clay—crop practically unaffected „ „ „ „
1924. Potatoes planted about 3 weeks after sulphur treatment.
Light Soil—crop little affected.
Clay—crop badly scorched.

1925. *Light Soil*—An application of 10 cwts. per acre only, either in the previous autumn or in the spring, caused an almost complete loss of crop. The plots when replanted still failed to produce a crop. Amongst the great majority of plants, which barely came through the soil and only bore a few small tubers of which the skins were brown, were a few well-grown plants.

The inference to be drawn from our experiments is that under certain conditions sulphur treatment results in at least one succeeding potato crop being practically

free from wart disease. We have shown that thoroughness of incorporation is a necessary condition, but there are certain others which require further investigation.

As to the mode of action of sulphur in the soil we are equally ignorant. I think that we have fairly good evidence that the action is not solely dependent on the acidity produced, though a slightly acid reaction seems to be necessary. This latter point is of interest in connection with Professor Barker's statement that the presence of a small amount of sulphuric acid on the sulphur particles is necessary for the full activity of the sulphur.

Dr Brierley (Rothamsted Experimental Station) said that the only personal experience he had had of the fungicidal use of sulphur was in connection with experiments on the control of wart disease of potatoes which had been done in collaboration with Miss Glynne, Dr Crowther and Mr Roach at Rothamsted. Since 1920 they had carried out extensive field tests, the sulphur and sulphur compounds being mixed intimately with the soil by means of a Simar Rotary Tiller. An account of much of the work had been published in the *Annals of Applied Biology* and Mr Roach had just indicated some of the conditions and complexities inherent in this research and had made mention of the discrepant results obtained this year. There appeared to be no doubt that finely ground sulphur had a marked toxic action on the fungus in the soil but, of the way in which it acted, we knew practically nothing and, from any practical standpoint, the results were greatly reduced in value by their uncertainty and the difficulty of interpreting them and by their discordant character from year to year. This appeared to be due to the almost infinite complexity and variability of the conditions under which the sulphur acted in the soil.

When experimenting with sulphur in glasshouses the conditions were relatively simple, only factors of a largely controllable nature such as temperature, light, moisture, etc., being involved. In the field, however, one was not only confronted with crop susceptibilities and English weather conditions but with all the physical and chemical conditions of the soil, and, perhaps still more vital, with a fluctuating and largely unknown living population of micro-organisms, many elements of which were able to utilise sulphur and, indeed, many other common fungicidal substances, as sources of food and energy.

Direct field studies were thus weighted with a number of unknown variables which, at times, seemed to be of sufficient value not only to nullify but completely to reverse particular results on the same soil in successive years. Such investigations were bound to be purely empirical and in the work that Dr Brierley and his colleagues had done they had tried, and to a certain extent succeeded, in making such an empirical attack on the wart disease problem using sulphur as the toxic agent. He was not, however, sanguine that much, if any further value, could come from this kind of work especially if the study of the fungicidal action of sulphur in soil were dependent upon the investigation of a problem of such peculiar difficulty and special character as wart disease of potatoes.

In the almost complete absence of knowledge of the nature and activities of soil organisms in relation to sulphur and sulphur compounds, and of the part that physical and chemical factors of the soil and climatic and crop factors may play in conditioning fungicidal action and biological interaction, only the empirical methods that Dr Brierley and his collaborators had used were available for work on a field

scale. Their results had shown that sulphur is a potent soil fungicide, and also, that its use as a soil fungicide is a problem of primary importance standing on its own merits quite apart from wart disease or any other specific study.

Dr Brierley was of opinion that empirical attacks on this purer issue would, owing to the great complexity of the problem, largely be a waste of time and effort and that valuable results or, indeed, any further advance in our knowledge, would only come from far-sighted co-ordinated researches of a fundamental nature in which physicist, chemist and micro-biologist, all of whom were equally concerned, would need to combine.

Mr Tattersfield (Rothamsted Experimental Station) considered that although the precise composition of lime sulphur could not be exactly specified, that most chemists would agree that the polysulphide content characterised it most accurately. Everybody concerned in either its manufacture or its use owed a debt to Dr Goodwin for his simplification of Chapin's method of estimating the polysulphide present, as even after a short experience a competent chemist could state how carefully the material had been made and how carefully stored.

With respect to the fungicidal action of the polysulphides it would definitely appear as if the direct effect were due to alkalinity and the polysulphide acting as such, but that the prolonged protective effect and the action at a distance were in some way bound up with sulphur particles deposited from the solution. This latter fact immersed them at once in a general discussion of sulphur as a fungicide.

It appeared to him that the bent tube experiment of Barker, Gimingham, and Wiltshire was of first-class importance, indicating as it undoubtedly did the importance of the part played by sulphur in the particulate form.

Solid materials dispersed in air in a very finely divided form were characterised by heightened chemical effects due to their enormously increased surface. Thus violent dust explosions could take place when combustible particles were finely dispersed in air and chemical reactions could be catalysed by their means. Such reactions were due partly to the fineness of division and the increased adsorption due to the greatly extended surface. Moreover, it was known that particles blown through air were liable to take on an electric charge.

It was questionable whether Young's ascription of the fungicidal action of sulphur to the polythionic acids was generally valid. Whether right or wrong, however, this view did not affect the significance of the part played by sulphur in a finely divided form, and it seemed wiser in the present state of our knowledge to refer its action as a fungicide to the heightened chemical effects due to the peculiar properties associated with its state of division.

Dr G. H. Coons (Michigan Agricultural College, U.S.A.) said: The symposium "Sulphur as a Fungicide" has been of great interest to me. It is very evident that the workers of England are investigating thoroughly this very important substance and your work in part is confirming and in part putting in question the contributions of my American colleagues.

I am not able to speak in a first hand way of sulphur as a fungicide—my connection with the investigations being only casual. It may be of interest to you to know the general scope of the work in America and its present status as well as to have reviewed the history of our sulphur investigations.

Following the discovery, by Professor A. B. Cordley of the Oregon Experiment Station, that lime sulphur, previously used only as a dormant spray, could be used in diluted form as a summer spray, the spray practice among fruit growers was almost revolutionised—lime sulphur almost completely replacing Bordeaux mixture. The reasons for this revolution in practice are to be found in the greater simplicity of preparation on the farm, the cheapness, the better quality of fruit, since russeting is uncommon with lime sulphur, and the fact that lime sulphur can be bought ready for use.

The work of Dr Errett Wallace showed that the fungicidal action of lime sulphur came from the calcium polysulphides and these eventually, on the leaf, deposit elemental sulphur, finely divided. As an interesting corollary of this work came the dusting programmes where finely divided sulphur was directly applied to the leaf. This work has been most ardently advocated by Whetzel and his associates at Cornell University.

For many years after this pioneer work, investigation of lime sulphur and of sulphur practically ceased, and it was only in the past three years that revival of investigational work began.

Through the efforts of the Crop Protection Institute, a Committee of the National Research Council, funds were obtained from the larger sulphur-producing companies in the United States and fundamental studies of sulphur as a fungicide and an insecticide begun. One of the products of this work is the report of Dr Young which has been referred to in this symposium. The other phase—insecticidal properties of sulphur—has been carried on at the Geneva Experiment Station and is being published in the *Journal of Economic Entomology*.

I may summarise the work on sulphur as a fungicide by saying that Dr Young suggests pentathionic acid as the fungicidal agent produced from sulphur. From his work and the work of other investigators, many new methods of producing colloidal sulphur—or at least finely divided sulphur have been discovered. The work of Dr Young is being continued by Dr L. K. Tisdale at the Missouri Botanic Garden and I am sure the later and more complete account of sulphur as a fungicide will be of great interest to English workers.

I may say also that by co-operation of five different Experiment Stations, field tests of these new compounds have been made, and these, while showing promise, have not as yet reached the final stage. The gap between the laboratory and the field is large and hard to bridge.

In conclusion, I may say that, be the fungicidal agent in sulphur preparations what it may, the general opinion of American investigators is (1) that better sulphur preparations are necessary for our fruit growers to avoid the burning, the leaf cast and leaf discoloration which accompanies the use of lime sulphur, (2) that the scientific work so far emphasises the point that fineness of presentation of the sulphur particles is necessary for greatest efficiency, and (3) that the investigations open up a most promising field of study.

I sincerely wish to thank the chairman for affording me this opportunity of meeting my English colleagues and I wish to congratulate you on the splendid research which you have inaugurated.

Mr C. T. Gimingham (Rothamsted Experimental Station) referred to the

experiments described by Professor Barker indicating the dispersion of sulphur in the air in the form of minute discrete particles and said that much work was done on the subject of particulate "clouds" during the war, especially with reference to organic arsenic compounds.

It is well known that some of these substances can be readily distributed in the air in particulate form. In attempting to determine the amount of material present in such "clouds," it was found that the concentration was greatly reduced by passage through a bent tube or even through a narrow straight tube, most of the particles being condensed on the walls of the tube. Further, the arsenic compound could be completely removed from the air by the use of appropriate dense filter pads. There is thus a very close parallelism between the behaviour of these substances dispersed in the air in particulate form and that of sulphur under certain conditions.

Experience of the physiological effect of even minute traces of organic arsenic compounds when dispersed as a particulate "cloud" indicates that matter in this form may possess very special properties which are not so evident under other conditions.

Dr G. H. Pethybridge (Ministry of Agriculture and Fisheries) called attention to the danger of assuming that to any given fungicide a definite, constant fungicidal value can be assigned. Chemists and physicists were accustomed to deal with constants such as melting and boiling points, indices of refraction and so on, but fungicidal value is not a constant of this kind. A particular substance may be lethal for a certain fungus at a certain concentration in a certain time and at a certain temperature, whereas, under similar conditions, the same substance may be without effect on another fungus. Furthermore, the reaction of one and the same fungus to a given fungicide may vary according to age and to the particular stage of development or life-history of the fungus at the moment of application; and different morphological parts—mycelium, spores, etc.—may also react differently. It seems probable also that the medium on which a fungus is growing may be of importance, in some cases, in relation to its power of resisting or succumbing to the action of poisons. Data obtained with cultures *in vitro* may not be applied without discrimination to parasites on their natural hosts. The influence of environment on the fungicide may in some cases be important. It does not necessarily follow that the action of sulphur, for instance, under sub-aerial conditions would be identical with its action under subterranean conditions. Such considerations, and others which might be advanced, show that caution is needed before wide generalisations on fungicidal values are formulated.

REPORT OF THE COUNCIL FOR THE YEAR 1925

During the year eight meetings of the Association have been held with an average attendance of 52 members and visitors. The provincial meeting was held in the University of Edinburgh and extended over two days; on both days there were large attendances and great interest was shown in the programme submitted by Members of the University staff and senior research students. The *Scotsman* gave each day a full report of the proceedings. Altogether the Edinburgh visit was a signal success, many admirable papers were read, interesting exhibits were shown and demonstrations were held; in addition several teaching and research centres were visited and places of historical interest. Everywhere members were met with the warmest hospitality. The best thanks of the Association are due to Professor J. H. Ashworth, Dr R. Stewart MacDougall, Professor Wright Smith and Dr Malcolm Wilson and their colleagues by whose energy and organisation the long two days' programme was carried through.

The summer meeting was held at the invitation of Messrs Bunyard in the nurseries of this firm at Maidstone, a very profitable day was spent; the thanks of the Association are due to Messrs Bunyard for their kind hospitality and excellent organisation of the meeting. Many interesting communications have been presented to the Association at its meetings in London, among the proceedings stand out a very excellent discussion on the "Use of Sulphur as a Fungicide" also a discussion on the "Place of the Systematist in Applied Biological Work." The Council mark with regret the deaths during the year of Dr Lilias Hamilton and Mr W. R. Dykes and a former and very prominent member, Professor H. Maxwell Lefroy. Eight new members have been elected during the year and there have been six resignations. The present roll of the Association includes the names of 12 Honorary Members and 231 Ordinary Members.

The thanks of the Association are due to the Authorities of the Imperial College for their kind permission to the Association to hold its meetings in the Botany Lecture Room at the College.

The following communications have been made to the Association:

Mr W. Rushton: "The Biology of the Jersey Water Works."

Professor J. C. Ewart: "The Production of Fine Wool."

Dr F. A. E. Crew: "Micrological Examination of the Fleece of different British Breeds of Sheep."

Dr F. A. E. Crew: "Intersexuality in Domesticated Mammals and Fowls."

Dr J. Ritchie: "The Control of Mussels in Sea-Water Pipes."

Major W. S. Patton: "The Diptera which produce Myiasis in Man and Animals."

Professor J. Russell Greig: "The possible Association of *Tilletia tritici* with Epileptiform Convulsions in the Dog."

Dr W. G. Smith: "The Relation between Hill Pasture and Sheep Grazing."

Mr W. Robb: "Experiments in the Hybridisation of Oats."

Mr J. W. Gregor: "Observations on the Physiology of Reproduction in some Agricultural Grasses."

Professor J. M. F. Drummond and Mr F. W. Sansome: "The Improvement of Swedes and Turnips by Breeding."

Dr R. Stewart MacDougall: "The Ox-Warble Flies (Hypoderma) and their Control."

Mr P. H. Grimshaw: "The Occurrence of *Hydrellia griseola*, a Continental Barley Pest, in Britain."

Mr J. N. Oldham: "*Hieroxestis subcervinella* as an enemy of the Banana."

Dr Malcolm Wilson: "*Rhizosphaera piceae* n.sp., causing Disease of Spruce Firs."

Dr Malcolm Wilson: "The Occurrence of *Tuberculina maxima* in Scotland and its Effect on the Blister Rust of the Weymouth Pine."

Mr A. Millar: "Post-War Progress in the Study of the Potato."

Mr T. Anderson: "Modern Aspects of the Practice of Seed Testing."

Dr Wm. Brierley: "The eradication of Wart Disease by means of soil sterilisation with particular reference to the use of Sulphur."

Dr E. M. Crowther: "Soil reaction in relation to sulphur treatment for Wart Disease."

Mary D. Glynne: Wart Disease of Potatoes (i) "Laboratory Testing for Immunity"; (ii) "Pot experiments in relation to soil sterilisation"; (iii) "Viability of winter sporangia."

Mr W. A. Roach: "Biochemical investigations of immunity to Wart Disease of Potatoes."

Mrs K. St-G. Cartwright: "On the nature of the Resistance of the Potato to Wart Disease."

Dr J. Davidson: "Factors affecting the Infestation of *Vicia faba* by *Aphis rumicis*."

Professor V. H Blackman and Dr W. Brown: "Change of Seed of Potatoes."

Professor E. S. Salmon: Opener of a discussion on "The Use of Sulphur as a Fungicide."

Dr J. Waterston: Opener of a discussion on "The Place of the Systematist in Applied Biological Work."

Dr A. D. Imms: "The Biological Control of Pests in the United States and Hawaiian Islands."

REPORT OF THE HON. TREASURER FOR THE YEAR 1925

A statement of the accounts of the Association for the year ending Dec. 31st, 1925, is given on p. 322. The expenses during the year have been considerably higher than during any previous year, which is accounted for as follows: Firstly, there is an increase of £132. 8*s*. 10*d*. in meeting the publisher's account for the *Annals of Applied Biology* as compared with the amount required for the previous year. Secondly, expenses incurred by the chief officers of the Association in attending country meetings have been met out of the funds of the Society. Thirdly, tea has been provided gratis to members attending each London meeting.

Income from current subscriptions amounted to £220. 9*s*. 0*d*. as compared with £258. 14*s*. 0*d*. for the previous year. This decrease is mainly owing to the fact that over 40 members are in arrears with their subscriptions, a larger number than has been the case in previous years. It is urgently requested that all members concerned will make a special effort to pay their contributions promptly and that those who are in arrears will clear off their debts to the Society as early as possible during the current year.

Receipts for trade subscriptions and sales of the *Annals* show an increase of £118. 15*s*. 4*d*. which is mainly due to an augmented demand for back volumes and for reprints of separate articles. This increase in trade receipts has resulted in a sum of £126. 11*s*. 9*d*. being required to meet the publisher's account for volume XII as compared with £303. 1*s*. 0*d*. for volume XI.

Our assets exceed our liabilities more than six times over and of the former £756. 13*s*. 6*d*. is represented by cash at the bank or invested in National Savings' Certificates. The position and future of the Association and its *Journal* may, therefore, be regarded as satisfactory from the financial standpoint.

TREASURER'S STATEMENT FOR THE YEAR ENDING 31ST DECEMBER, 1925

CASH ACCOUNT.

Cr	£	*s.*	*d.*	*Dr*	£	*s.*	*d.*
Jan. 1. Cash at Bank . . .	129	17	5	Postage	7	5	9
Dec. 31. Subscriptions:				Stationery and minor printing	10	7	0
A. Current . . .	220	9	0	Treasurer	309	4	7
B. Arrears . . .	7	10	0	Secretaries	15	3	9
C. Advances . .	4	0	0	Balance at Bank	70	8	6
Entrance Fees . .	4	14	6	Placed on Deposit	130	0	0
Contributions to cost of papers in *Annals*	22	0	3				
From Deposit . . .	150	0	0				
Bank Interest . . .	3	18	5				
Total	£542	9	7	Total	£542	9	7

BALANCE SHEET.

Liabilities.	£	*s.*	*d.*	Assets.	£	*s.*	*d.*
Subscriptions in advance . .	4	0	0	Current a/c	70	8	6
Liability on *Annals*, vol. XII .	126	11	9	Deposit a/c	230	0	0
Excess of assets over liabilities	729	1	3	Subscriptions two years or less in arrears and considered good	44	2	0
				National Savings' Certificates.	456	5	0
				Estimated value of stock of *Annals of Applied Biology* with publishers	58	17	6
Total	£859	13	0	Total	£859	13	0

A. D. IMMS, *Hon. Treasurer.*

We have examined the Treasurer's statement of expenditure and receipts and have found it correct. We consider that the above balance sheet correctly represents the position of the Association.

J. C. F. FRYER.
R. STENTON.